Maternal Overweight and Obesity: Effect on Fetal Growth and Adiposity

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Glossary of Terms

AA	Abdominal area
AC	Abdominal circumference
ACOG	American College of Obstetricians and Gynecologists
ADIPS	Australian Diabetes in Pregnancy Society
AFI	Amniotic fluid index
AFM	Abdominal fat mass
BMI	Body mass index
BPD	Biparietal diameter
CI	Confidence interval
EFW	Estimated fetal weight
FL	Femur length
FMC	Flinders Medical Centre
GDM	Gestational diabetes mellitus
GEE	Generalised estimating equations
GRADE	Grades of recommendation assessment, development and evaluation
HC	Head circumference
HDL	High density lipoprotein
IADPSG	International Association of the Diabetes and Pregnancy Study Groups
IL-6	Interleukin-6
IQR	Interquartile range
IRSD	Index of Relative Socio-economic Disadvantage
LGA	Large for gestational age
LMH	Lyell McEwin Hospital
MTFM	Mid-thigh fat mass
MTLM	Mid-thigh lean mass
MTTM	Mid-thigh total mass
NICE	National Institute of Clinical Excellence
NICU	Neonatal intensive care unit
OGCT	Oral glucose challenge test

OGTT	Oral glucose tolerance test
OR	Odds ratio
PCOS	Polycystic ovary syndrome
SD	Standard deviation
SEIFA	Socio-Economic Indexes for Areas
SGA	Small for gestational age
SSFM	Subscapular fat mass
TNF-α	Tumour necrosis factor-alpha
TOBEC	Total body electrical conductivity
VLDL	Very low density lipoprotein
VTE	Venous thromboembolism
WCH	Women's and Children's Hospital
WHO	World Health Organisation

Abstract

Background

Maternal overweight and obesity are associated with well-documented maternal and infant risks, including high infant birth weight and delivery of an infant large for gestational age. However, less is known about the impact of maternal BMI on fetal growth and adiposity, and growth and adiposity trajectories. There is limited information regarding the effects of antenatal interventions to limit gestational weight gain on fetal growth and adiposity.

Methods

This thesis examines the effect of maternal BMI on fetal growth and adiposity, antenatal contributors to fetal growth and adiposity, and the effect of antenatal interventions on fetal growth and adiposity using data from a set of three harmonised randomised trials conducted between June 2008 and April 2017. Women were invited to attend for a research ultrasound at 28 and 36 weeks of gestation, where fetal biometry and adiposity measures were obtained. The analyses reported in this thesis investigate:

1. The effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity;
2. Whether this effect is mediated by (diagnosed and treated) GDM;
3. How fetal growth and adiposity is altered among infants born LGA; and
4. The effect of antenatal interventions to limit gestational weight gain on fetal growth and adiposity.

Results

The analyses reported in this thesis find that:

1. Maternal BMI exerts a strong, continuous positive effect on fetal growth and adiposity measures, from as early as 20 weeks' gestation;

2. Among women who are overweight or obese, there is no evidence of a mediated effect by diagnosed and treated GDM;
3. Infants born LGA demonstrate larger fetal biometry and adiposity measures from as early as 20 weeks' gestation; and
4. There is no evidence that the antenatal interventions investigated in this thesis are sufficient to alter fetal growth and adiposity.

Conclusions

Overall, the findings presented in this thesis suggest fetal growth patterns are determined early in pregnancy, and any antenatal interventions to prevent the effects of maternal overweight and obesity on fetal growth will need to be commenced earlier in pregnancy, or prior to conception, to be effective in preventing the intergenerational inheritance of overweight and obesity.

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.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

I acknowledge the support I have received for my research through the provision of an Australian Government Research Training Scholarship.

Amanda Josephine Poprzeczny
August, 2022

Acknowledgements

First and foremost, I am indebted to the women who participated in the LIMIT, GRoW and Optimise randomised trials. These women freely committed their time to the conduct of these trials, and without their generous involvement, this thesis would not have been possible.

It is insufficient to simply say "thank you" to Professor Jodie Dodd. Jodie is an exceptional clinical academic, constantly dedicated to the rigorous performance of clinical trials and application of evidence based medicine. You have taught me so much over the time of my PhD, and helped me commence a career in academic medicine. I have so appreciated your time, effort, advice and example, and I look forward to continuing to work together in the future.

A sincere thank you to Rosalie Grivell – without you encouraging me to commence and enrol in a PhD, this thesis would not be here. Your support and enthusiasm, particularly at the beginning of my PhD, started me down this road, and I will be forever grateful for that.

This thesis would not be here without the efforts and contributions of Jennie Louise. Jennie, you have taught me so much about critical thinking, biostatistics, and writing. Thank you so much for your encouragement, support, and work to help complete this thesis.

A wholehearted thank you to Andrea Deussen – you have been an invaluable support and sounding board through all stages of my PhD. I have so appreciated your time, thoughts, chats, and friendship. Thank you very much.

To the staff of the Lifelong Health Research Group – thank you so much for your friendship, support and encouragement over the last 5 years. The work you do

running the trials is invaluable, and I greatly appreciated how you welcomed and included me from the first day.

I could not have completed this thesis without the financial support I received from the RANZCOG Norman Beischer Clinical Research Scholarship, and the Avant Doctors-in-Training Research Scholarship. The recognition of the importance of clinical research in obstetrics, and support for the work I was doing, was crucial to my progress and completion.

To my long-suffering friends and family – I did it! Thank you for your understanding, love and support. Thank you to my parents, for teaching me always to question, and encouraging a career in academia. My family has grown and developed over the course of completing my PhD. To David and Thomas - I love you both so very much, thank you for being my family, for loving and supporting me in all the many crazy things I do. I can't wait to see what the future brings for us all, and am so glad we get to do it together.

Statement by Candidate

During my PhD candidature, I have been involved in the study design, the submission of any amendments to ethics, conduct of the research ultrasound examinations for fetal growth at 28 and 36 weeks' gestation, the collection of the ultrasound related data, and development of the statistical analysis plans under the supervision of my PhD supervisors. I was responsible for obtaining salary support through the RANZCOG Research Foundation and Avant Medical Indemnity Doctor in Training Research Scholarship Program. I was involved in the intellectual development and conduct of the exploratory analyses of the LIMIT, GRoW and Optimise randomised trials presented in this thesis, with two manuscripts published, and two currently under peer review.

Purpose and Scope

The purpose of this thesis is to examine the effect of maternal overweight and obesity on fetal growth and body composition, as well as the effect of antenatal interventions aimed to improve outcomes in women who are overweight or obese. This literature review will identify gaps in current knowledge, and form the background for the prospective studies that follow.

When reviewing the literature, a systematic search strategy was applied using the following key words and headings: Fetal development, fetal growth, fetal programming, fetal organ maturity, fetal wellbeing, fetal macrosomia, macrosomic, large for gestational age, adiposity, body composition, fat distribution, obesity, overweight, pregnancy, maternal obesity, maternal overweight, high BMI, elevated BMI, prenatal ultrasonography, ultrasound. The studies reviewed were assessed in keeping with the GRADE working group guidelines [1].

Chapter 1: Literature Review

1.1 Overweight and Obesity

1.1.1 What is overweight and obesity?

Overweight and obesity are defined as "abnormal or excessive fat accumulation that may impair health"[2, 3]. Among adults, overweight and obesity are most commonly classified using body mass index (BMI), which is a weight-for-height index, and is calculated as a person's weight in kilograms divided by the square of their height in metres (kg/m^2)[2, 3]. Using this ratio, people are classified as underweight (BMI <18.5 kg/m^2), normal weight (BMI 18.5 – 24.9 kg/m^2), overweight or pre-obese (BMI 25.0 – 29.9 kg/m^2), or obese (BMI ≥30.0 kg/m^2)[2, 3]. Obesity can be further subdivided into obese class I (BMI 30.0 – 34.9 kg/m^2), obese class II (BMI 35.0 – 39.9 kg/m^2), and obese class III (BMI ≥40.0 kg/m^2)[2, 3].

While useful as a simple classification and measure of excess body weight at a population level, there are limitations to using BMI[3-5]. The distribution of excess fat is an important determinant of risk of disease, with central or abdominal adiposity a strong risk factor for cardiovascular disease and diabetes[6, 7]. Measures of excess fat that take into account body fat distribution include waist circumference, waist:hip ratio and waist:height ratio[8]. Different ethnic groups demonstrate different body fat distributions for the same BMI. For example, compared with European Australians, Indigenous Australians have higher percentage body fat at the same BMI[9]. This may be important for risk identification. In children, BMI varies with age, and BMI-for-age percentiles are used to define overweight and obesity in this population[2, 3, 10].

Despite these limitations, BMI is the most accessible and commonly used measure of excess fat, and is considered the most useful population-level measure of obesity[2, 3, 11].

1.1.2 Scope of the problem

Overweight and obesity represent a significant global public health issue. Worldwide estimates suggest almost 40% of adult men and women, and almost 25% of children, are overweight or obese[12, 13]. In 2016, it was estimated that more than 1.3 billion adults were overweight and at least 670 million were obese[14]. This represents a near tripling of the prevalence of obesity over the last 40 years[15]. In Australia, 67% of all adults, and 25% of all children and adolescents, are overweight or obese[3, 16] . This is similar to other developed countries, with approximately 68% of adults in the United States[17, 18] and United Kingdom[19] being overweight or obese. While the greatest prevalence of overweight and obesity is in developed countries, it is increasingly recognised as a problem in developing countries[12, 13], representing a "double burden of malnutrition", associated with coexistence of overweight and obesity and significant undernutrition[20].

1.1.3 Health economics of overweight and obesity

Overweight and obesity represent a significant cost to the community. A survey of healthcare spending in the United States in 2006 estimated that overweight and obesity accounted for 9% of the country's total healthcare expenditure[21]. At an individual level, obesity confers an estimated 30% increase in health care costs when compared with patients who are not obese[22]. The Australian Longitudinal Study on Women's Health reported that overweight or obesity among women between the ages of 50 and 55 were associated with progressively increasing Medicare costs[23].

Data from the Australian Diabetes, Obesity and Lifestyle (AusDiab) study estimated that the total direct cost associated with overweight and obesity in Australia in 2005 was $18.8 billion ($10.5 billion for those who were overweight, and $8.3 billion for those who were obese), with a further $32.3 billion spent in government subsidies[24].

1.1.4 Risks associated with overweight and obesity

The health effects of overweight and obesity are considerable, and contribute to increased morbidity and mortality[11, 25, 26]. The Australian Burden of Disease Study reported overweight and obesity as the leading contributor to non-fatal disease burden in 2015[27]. Globally, the major contributors to increased mortality among people who are overweight or obese are cardiovascular disease and malignancy[28, 29].

1.1.5 Overweight and obesity and women's health

Women's health can be significantly impacted by overweight and obesity. Among adolescents, overweight and obesity are associated with earlier onset of puberty[30], and increased rates of menstrual disturbances[31]. Overweight and obesity can also affect fertility, reflected by a longer time to conception when compared with women of a normal BMI[32, 33], even in the setting of regular menstrual cycles[34]. When accessing fertility services, women who are overweight or obese also have lower rates of successful conception compared with women of a normal BMI[35, 36].

Women who are overweight or obese are at an increased risk of polycystic ovary syndrome (PCOS), with up to 28.3% of women fulfilling the diagnostic criteria for PCOS, compared with only 5.5% of lean women[37]. Polycystic ovary syndrome is an endocrine disorder characterised by hyperandrogenism, hyperglycaemia, and infertility. Longer term, women with PCOS are recognised to have an increased risk of developing both type 2 diabetes and cardiovascular disease[38].

Overweight and obesity are associated with multiple female-specific cancers. This includes a 1.45-fold increase in endometrial cancer for every 5 unit increase in BMI[39], and a doubling in the risk of developing cervical adenocarcinoma[40, 41]. The impact of overweight and obesity on risk of ovarian cancer is less clear,

although some have reported a 27% increased risk when compared with women of a normal BMI[42].

1.2 Overweight and obesity during pregnancy and childbirth

1.2.1 Scope of the problem

Overweight and obesity in pregnancy are defined most commonly using BMI assessed in early pregnancy. Other measurements used for defining excess adiposity, overweight and obesity in non-pregnant adults – including waist circumference, waist-hip and waist-height ratio – have limitations in pregnancy due to the impact of the developing fetus on maternal girth. It is recommended that pregnant women have their weight and height measured, and BMI calculated, as early in pregnancy as possible as part of routine antenatal care[43]. Maternal weight has been shown to vary little over the first trimester of pregnancy[44, 45], meaning early pregnancy BMI closely approximates pre-pregnancy measurements. Maternal self-reported height and weight systematically underestimates weight and overestimates height, resulting in up to 13% of women being misclassified with regards to BMI category[46]. In particular, error in self-reported maternal weight has been shown to be greater among women who are overweight and obese[46-48].

Globally, rates of maternal overweight and obesity are increasing. In the United Kingdom, rates have significantly increased between 1990 and 2004 (31.4% to 41.3%)[49]. Most recently, 22.2% of women entering pregnancy in the United Kingdom had a BMI ≥30.0 kg/m^2[50]. In the United States, maternal obesity has increased by 70% between 1993 and 2003 (13.0% to 22.0%)[51]. More recent examination of trends in maternal obesity in the United States suggest it continues to increase, with 26.1% of women entering pregnancy with a BMI ≥30.0 kg/m^2 in 2016, and 29.0% in 2019[52].

Australian data indicate that 47.5% of women entering pregnancy are overweight or obese[53]. While this varies slightly by state, South Australian

figures similarly identify 52.0% of women entering pregnancy overweight or obese[54]. Nationwide, rates of maternal Class II and III obesity have significantly increased between 1998 and 2009, from 3.7% to 5.2%[55].

1.2.2 Risks associated with maternal overweight and obesity

Overweight and obesity in pregnancy confer higher risk of many adverse outcomes to the woman and infant throughout the antepartum, intrapartum, and postpartum periods, all of which increase with increasing maternal BMI[56-58]. These risks, and estimates of the magnitude of increased risk compared with women of a normal BMI in pregnancy, are summarised in Table 1.1 below.

Table 1.1 – Risk of adverse maternal outcomes in overweight and obese pregnant women compared with women of a normal BMI

Outcome	Estimate of Increased Risk	References
Miscarriage	1.7x	(42, 59)
Gestational Diabetes	7.5x	(42, 56, 57, 60)
Hypertensive disorders of pregnancy	4.9x	(42, 56, 57, 60)
Stillbirth	2.0x	(58, 61)
Induction of labour	2.0x	(56, 58, 62)
Assisted vaginal delivery	1.3x	(42, 56, 63)
Caesarean section	2.5x	(42, 56, 57, 60, 62, 64)
Postpartum haemorrhage	3.0x	(58)
Postnatal infection	6.0x	(58)
Venous thromboembolism	5.0x	(65)

Women who are overweight or obese are at an increased risk of suffering a miscarriage, with an OR of 1.67 (95% CI 1.25-2.25) when compared to women of a normal BMI[42, 59].

The risk of both pre-existing type 2 diabetes and gestational diabetes mellitus (GDM) is progressively higher with increasing BMI[42, 56, 57]. This likely reflects the fact that there is a similar metabolic milieu observed among women who are overweight or obese and those with pre-existing type 2 diabetes or GDM, made up of varying degrees of insulin resistance, hyperglycaemia, hyperlipidaemia, and low-grade chronic inflammation[66]. Pregnancy is a state of increasing insulin resistance, and this contributes to increasing blood glucose concentrations and insulin requirements among women with pre-existing diabetes, or a diagnosis of GDM among women who do not have pre-existing diabetes[67, 68]. In an Australian cohort, almost 3% of pregnant women who were morbidly obese had type 2 diabetes, which was significantly higher than among women who were of a normal BMI in pregnancy[57]. Rates of GDM as high as 20% have been reported in women with a BMI $\geq$40 kg/m^2[69].

Women who are overweight or obese are at an increased risk of developing hypertensive disorders of pregnancy, including gestational hypertension and pre-eclampsia[56-58, 63]. Compared with women of a normal BMI, having a BMI $\geq$40 kg/m^2 is associated with a 6-times greater risk of developing pre-eclampsia[58]. There are multiple theories as to the etiology underlying this increased risk, including pregnancy-related exacerbation of underlying insulin resistance among women who are overweight or obese, the low-grade chronic inflammation associated with overweight and obesity, and genetic predisposition[70].

Maternal overweight and obesity are associated with a doubling of the risk of stillbirth (overweight vs normal BMI, OR 1.47, 95% CI 1.08-1.94; obese vs normal BMI, OR 2.07, 95% CI 1.59-2.74)[71], representing one of the largest population-attributable risk factors in high-income countries[72]. This association

is particularly strong for term stillbirth, the risk being 4 times higher among obese women compared with women of a normal BMI[73].

Reflecting an increased risk of pregnancy complications such as pre-eclampsia and GDM, women who are overweight or obese are more likely to require induction of labour[56, 58, 62]. Women who are overweight or obese are less likely to achieve spontaneous vaginal birth, with a converse increase in the rates of both assisted vaginal and caesarean birth[56-58]. Furthermore, maternal overweight and obesity increase a woman's risk for both elective and emergency caesarean birth[56, 62, 74]. Among women attempting a vaginal birth after caesarean section, maternal overweight and obesity contribute to an increasing risk of unsuccessful trial of labour and uterine rupture or scar dehiscence [75].

Associations between maternal overweight and obesity, and preterm birth, are less clear[57, 74, 76-78]. While maternal overweight and obesity have been associated with a decreased risk of spontaneous preterm birth compared with women of normal BMI[79], indicated or induced preterm birth were increased[79]. Thus, it is likely that adverse pregnancy outcomes such as GDM and hypertensive disorders of pregnancy substantially contribute to the risk of preterm birth in this cohort of women.

Women who are overweight or obese are twice as likely to require antenatal hospital admission and have an increased length of stay at the time of birth[57, 80, 81]. This represents a significant burden not only on women and their families, but also on healthcare systems, contributing to increased healthcare expenditure. Australian data indicates an additional 5 million dollars in healthcare costs over a calendar year can be attributed to maternal obesity, mainly as a result of increased maternal hospitalisation and length of stay after birth[82].

In the postnatal period, women who are overweight or obese have a three times higher risk of postpartum haemorrhage[58]. The risk of infection is also increased[74], as is the risk of postpartum venous thromboembolism (VTE)[65],

reflecting a combination of the chronic inflammatory state associated with obesity, longer duration of labour and increased risk of operative birth and infection.

1.2.2.2 Fetal and neonatal risks associated with maternal overweight and obesity

Infants born to women who are overweight or obese are also at increased risk of adverse birth outcomes. Increasing maternal BMI represents a significant independent risk factor for the diagnosis of a fetal congenital anomaly[57, 83], particularly the risk of neural tube defects (3-fold)[84], and congenital heart defect (1.4-fold)[85]. It has been hypothesised that the abnormal metabolic environment associated with overweight and obesity may contribute[86]. Increasing maternal BMI is associated with increasing technical limitations of antenatal ultrasound, and potentially reduced detection rates of fetal congenital anomalies[87, 88].

Infants born to women who are overweight or obese are at an increased risk of a range of adverse neonatal outcomes, summarised in Table 1.2. Increasing maternal BMI is a well-recognised continuous risk factor for birth of a large for gestational age (LGA), or macrosomic, infant[56, 63, 74, 89]. Among women with BMI ≥40 kg/m^2 the risk of an infant being born LGA is increased almost 4-fold, compared with women of a normal BMI[63]. High infant birth weight also increases the risk of shoulder dystocia and birth trauma[63, 90]. Conversely, the risk of delivering an infant small for gestational age (SGA) is reduced among women who are overweight or obese[74].

At birth, infants born to women who are overweight or obese are at increased risk of low Apgar scores[56, 63, 74], and need for admission to the neonatal intensive care unit[56, 57, 74]. Infants are more likely to have neonatal hypoglycaemia, reflecting the associations with both maternal diabetes and high infant birthweight[57, 81].

Overall, additional costs for infant care associated with maternal overweight and obesity are estimated in the order of 1.1 million dollars per year[82], reflecting predominantly increased nursery admission[82].

Table 1.2 – Risk of adverse fetal and neonatal outcomes in overweight and obese women compared with women of a normal BMI

Outcome	Estimate of increased risk	References
Neural tube defect	3.0x	(57, 83, 84)
Congenital heart disease	1.4x	(57, 83, 85)
Large for gestational age/macrosomia	4.0x	(56, 63, 74, 89, 91)
NICU admission	1.8x	(56, 61, 74, 89)
Neonatal hypoglycaemia	7.0x	(57, 81)

In the longer term, maternal overweight and obesity are independent risk factors for childhood obesity[92-94]. This may be partially mediated by high infant birth weight, which is itself associated with future risk of both childhood and adolescent obesity, and may lie on the causal pathway between maternal overweight and obesity and childhood obesity[95].

1.3 Large for gestational age infants

1.3.1 What is large for gestational age?

Large for gestational age (LGA) infants are variably defined as those with a birthweight greater than the 90th, 95th, or 99th percentile for gestational age and sex. The term LGA is sometimes used interchangeably with "macrosomia"[96]. Most commonly it is used to define those infants with birthweight $\geq$90th percentile for gestational age and sex. In comparison, "macrosomia" refers to infant birthweight above a specific threshold, irrespective of gestational age. Most commonly it is used to refer to infant birthweight >4000gm or >4500gm[97]. Concomitant with increasing rates of maternal overweight and obesity worldwide, there are similar increasing trends in the birth of infants large for gestational age[98-101].

Women who birth an LGA infant are at increased risk of caesarean section, and postpartum haemorrhage[102-105]. Infants born LGA are at an increased risk of both short and longer-term adverse outcomes. These risks, and estimates of the magnitude of increased risk compared with infants not born LGA, are summarised in Table 1.3 below.

Table 1.3 – Risk of adverse outcomes in infants born LGA compared with infants not born LGA.

Outcome	Estimate of increased risk	References
Shoulder dystocia	2.6x	(102)
Neonatal hypoglycaemia	2.5x	(102)
Childhood and adolescent obesity	2.2-4.6x	(106, 107)
Adult obesity	1.3x	(108)
Type 2 diabetes mellitus	1.9x	(108)

In the short term, infants born LGA are at an increased risk of shoulder dystocia, birth trauma and neonatal hypoglycaemia[102]. In the longer term, infants born LGA are at increased risk of childhood and adolescent obesity[106, 107] and type 2 diabetes[108].

1.4 Fetal growth and adiposity

1.4.1 The importance of the fetal period

An understanding of fetal growth, and the effects of maternal overweight and obesity on fetal growth, may allow for better targeting of interventions to prevent later metabolic effects. Almost 30 years ago, David Barker proposed the developmental origins of adult health and disease hypothesis[109]. Under this hypothesis, maternal nutrition during pregnancy "programs" a phenotype that predisposes to an increased risk of future ill-health[109]. In the setting of gestational diabetes and maternal overweight and obesity, it is hypothesised that maternal hyperinsulinaemia stimulates fetal overgrowth and adipose tissue deposition[110]. While initially the focus was on the health effects related to intrauterine fetal growth restriction and low birth weight, there has been considerable interest in the longer term effects of maternal overweight and obesity and GDM, and high infant birth weight[111].

1.4.2 Fetal and neonatal adiposity

There is growing interest in measurement of fetal and neonatal body composition, as a more sensitive measure of adiposity. Among adults, it is acknowledged that measures reflecting central adiposity are more sensitive predictors of metabolic dysregulation and future cardiometabolic disease[6]. Whether fetal and neonatal measures of relative fat distribution, and relative fat and lean mass, more sensitively represent the effects of an intrauterine environment disordered by maternal overweight and obesity remains to be determined.

It has been proposed that neonatal lean body mass is predominantly determined by individual genetics, and that neonatal fat mass is influenced by the variable maternal environment[112, 113]. In reality, it is likely that the relationships between fetal and neonatal lean and fat mass, and the effects of dysregulated intrauterine environments such as those in maternal overweight and obesity are highly complex and inter-related.

1.4.3 Physiology of fetal fat deposition

At birth, the neonate is composed of 12-15% body fat mass, the greatest of any species[114]. The vast majority of accumulation and growth of fetal fat occurs in the third trimester of pregnancy, with rates of fetal fat deposition reaching 7 grams per day near term[114]. Stem cell precursors of adipose cells can become either brown or white adipose tissue[115], with first, predominantly white adipose tissue being deposited from mid to late gestation, and then mainly brown adipose tissue in the late third trimester, in preparation for birth[116]. The human neonate is born with large deposits of brown adipose tissue, which is vital for non-shivering thermogenesis[115]. Over time, these deposits of brown adipose tissue are lost, and white adipose tissue deposits grow, representing a site for energy storage and production of hormones important for appetite regulation and energy homeostasis[115].

Fat mass at birth is a significant determinant of overall body weight, accounting for approximately 46% of the variance in birthweight[117]. As such, measuring fetal and neonatal fat mass, and determining factors that contribute to their deposition, represents an important area of research.

1.4.4 Measuring fetal and neonatal adiposity

Multiple ultrasound measures of fetal fat mass have been developed, although most have been evaluated only in small studies. Furthermore, assessments have been focused on fetuses of women with diabetes, and often only at single time points in the third trimester of pregnancy rather than serially. Data relating fetal ultrasound measures with neonatal fat mass is contradictory.

Fetal adiposity measures most frequently examined include abdominal wall fat mass, mid-thigh total mass, mid-thigh lean mass, mid-thigh fat mass, and subscapular fat mass. These measures have been validated and are reproducible across different populations[118-121]. Abdominal wall fat mass is a measurement of the anterior subcutaneous tissue at the level of the abdominal circumference[119-121]. Mid-thigh total, lean and fat mass measurements are obtained on a cross-sectional view of the mid-shaft of the fetal femur. Mid-thigh fat mass is obtained by subtracting the central lean limb area (consisting of muscle and bone) from the total cross-sectional limb area[118-120].

Bernstein and colleagues investigated the pattern of growth of fetal mid-thigh and mid-arm fat areas over the third trimester of pregnancy[122]. This study included 36 women between 34 and 40 weeks' gestation, all of normal BMI. Both mid-thigh and mid-arm fat areas increased with increasing gestation, with increasing rates of fat accretion occurring later in the third trimester[122]. Similarly, Hill and colleagues demonstrated that mid-thigh fat mass and anterior abdominal wall fat mass measurements all increased between 24 and 41 weeks' gestation[123]. However, the rate of change and reference ranges for these fetal adiposity measures by gestation was not reported.

Larciprete and colleagues defined gestation-specific reference ranges for fetal subcutaneous tissue thickness measurements in an Italian population, and also considered the effect of GDM on fetal adiposity[119]. This study included 303 women, of whom 85 were diagnosed with GDM[119]. The generated reference ranges from 20 to 40 weeks' gestation demonstrated that measures of fetal adiposity increase over pregnancy, with the greatest rate of growth occurring in the late third trimester[119]. In women diagnosed with GDM, fetal subcutaneous tissue thickness measurements at multiple time points over pregnancy were greater when compared with women who did not have GDM[119]. While this is one of the largest populations in which serial measurement of fetal adiposity were performed, the generalisability of the findings is limited. Women enrolled in this study were predominantly of a normal BMI, and were recruited from a

group of women considered to be at higher risk for development of GDM based on clinical risk factors.

Measures of infant body composition may represent a more sensitive measure of future risk of childhood obesity and cardiometabolic dysfunction than birthweight alone[124]. Infant body composition and adiposity has been assessed using skinfold thickness measurements, air-displacement plethysmography, and total-body electrical conductivity (TOBEC). Routine ultrasound measures of fetal biometry have thus far only poorly correlated with neonatal adiposity measures[125, 126], however less is known about the association between ultrasound measures of fetal adiposity and neonatal adiposity measures. While an ability to accurately identify antenatally the fetus at risk of an adverse body-fat distribution and future obesity would allow for early recognition and intervention, further development of accurate tools and appropriate assessment is required.

O'Connor and colleagues conducted a prospective longitudinal study investigating the relationship between third trimester ultrasound fetal subcutaneous tissue measurements and neonatal body composition measured by air displacement plethysmography[127]. This study involved 62 women with both pre-existing diabetes mellitus and GDM[127]. The mean maternal BMI of the cohort was 27.5 kg/m^2[127], however the distribution of women within each BMI category is unclear. Fetal subcutaneous tissue measurements included anterior abdominal wall thickness, and thigh muscle and fat thickness, assessed serially from 28 to 38 weeks' gestation[127]. Although a significant positive correlation between both anterior abdominal wall thickness and thigh fat thickness and infant fat mass at birth was identified, it involved only measurements obtained at 38 weeks' gestation, and not at earlier gestational ages[127]. While the full range of fetal subcutaneous tissue thickness measurements were not evaluated[118, 119], the findings do indicate that measures obtained late in the third trimester correlate with infant body composition.

In contrast, Moore and colleagues showed that fetal adiposity measures performed at 28 weeks' gestation had a moderate-strong positive correlation with neonatal percent body fat and skinfolds[128]. This was a relatively small study of 44 women, and utilised 3D fractional limb volumes of the fetal thigh and upper arm. While this method may provide greater accuracy, the process requires greater technical training and is time-consuming in clinical practice, limiting routine clinical application[128].

Anterior abdominal wall and mid-thigh tissue fat thickness measures in the third trimester have been well correlated with their respective skinfold performed within 72 hours of birth[129]. The mean time between research ultrasound and performance of neonatal skinfold measurement was 10 days. Again, this significantly limits opportunity for antenatal intervention or modification of infant risk.

Overall, late pregnancy ultrasound measures of fetal adiposity correlate somewhat with neonatal adiposity, however further evaluation is required. Of note, these results should be validated in a larger, contemporary Australian population, including women who are overweight or obese.

1.4.5 Effect of maternal overweight and obesity on fetal and neonatal adiposity

There is limited evidence to suggest that infants born to women who are overweight or obese have greater fat mass and percentage body fat than infants born to women with a normal BMI[112, 130]. Sewell and colleagues identified that overweight or obese women were more likely to give birth to an infant weighing more than 4kg[112]. While infants were found to have an increased fat mass and percent body fat, as determined by TOBEC, there were no differences in lean body mass[112]. The authors hypothesised that the well recognised increased risk of infant macrosomia associated with maternal overweight and obesity was potentially as a result of increased neonatal fat mass.

Hull and colleagues performed a similar analysis using air-displacement plethysmography to measure neonatal fat and fat-free mass[130]. Although the proportion of women with a BMI ≥25 kg/m^2 was small, and the infants were up to 35 days of age at assessment, there were no significant differences in birthweight. However, infants of women who were overweight or obese were found to have greater body fat and fat mass[130].

The Healthy Start Study involved 826 women, of whom 369 were overweight or obese in early pregnancy[131]. Maternal BMI was positively associated with neonatal fat mass and percentage body fat as assessed by air displacement plethysmography performed within 72 hours of birth. Specifically, with every 1 kg/m^2 increase in maternal BMI there was an average 5.2 gm (95% CI 3.5-6.9 gm) increase in neonatal fat mass [131].

While these findings suggest maternal overweight and obesity are significantly and positively related to neonatal adiposity measures, less information is available describing the effect of maternal overweight and obesity on fetal measures of adiposity, and whether they can be modified in the antenatal period by interventions such as diet and lifestyle advice, or metformin.

1.4.6 Fetal growth and adiposity among infants born LGA

There are some data on fetal growth and adiposity, and fetal growth trajectories, of infants born LGA to women who do not have pre-existing diabetes mellitus. Madendag and colleagues reported the ultrasound findings from 582 women without diabetes which were obtained between 26 and 28 weeks' gestation[132]. All women had a BMI <30 kg/m^2. Mean fetal AC, FL measurements, and calculated EFW, were significantly greater among infants who went on to be born LGA[132]. Anterior abdominal fat mass measurements were also obtained, and were significantly greater among infants born LGA[132]. The findings of this work are limited with relatively few (64) LGA infants in the cohort. Furthermore, only a single time point for ultrasound was considered, precluding evaluation of fetal biometry and adiposity measure trajectories over pregnancy.

Caradeux and colleagues evaluated longitudinal measurements of AC z-scores over the second and third trimesters to predict LGA status[133]. Both higher fetal AC z-scores and AC z-score velocity were associated with birth of an infant LGA[133]. While this study included 2,696 women who did not have pre-existing diabetes[133], other fetal biometry measures were not reported.

Together, these data suggest that infants born LGA may have altered fetal growth, particularly in the third trimester of pregnancy. When during pregnancy the fetal growth of infants born LGA differs from those infants born AGA, and whether these differences are seen in all, or just some, ultrasound measures of fetal biometry and adiposity, is not known.

1.5 Determinants of fetal growth

Fetal growth is regulated and determined by a complex interplay of genetics, maternal nutrition, placental function, and events occurring over pregnancy. While there has been significant interest in the underlying mechanisms involved in fetal growth restriction, less is known about the determinants of and mechanisms involved in LGA fetuses and infants.

1.5.1 Hyperglycaemia and gestational diabetes mellitus

Glucose was first proposed as a primary fuel substrate for fetal overgrowth in 1967, when it was recognised that women with pre-existing diabetes mellitus in pregnancy were more likely to birth infants who were macrosomic, or LGA[134, 135]. Under the Pedersen hypothesis[134], maternal hyperglycaemia increases placental glucose transport, resulting in stimulation of insulin and other growth factor production from the fetal pancreas, which in turn stimulates increased fetal growth and fat deposition[134].

Gestational Diabetes Mellitus (GDM) is defined as "any degree of glucose intolerance with onset or first recognition during pregnancy" [136-138]. While this

condition has been recognised for many years[139, 140], significant controversy has existed regarding the benefits of screening, testing, diagnosing, and treating hyperglycaemia less severe than overt diabetes mellitus in pregnancy, as well as the diagnostic criterion used. The ACHOIS study[141] recruited 1000 women who had one or more risk factors for GDM on selective screening or a positive oral glucose challenge test (non-fasting 50g glucose load with 1 hour glucose level ≥7.8 mmol/L), and had a positive oral glucose tolerance test performed between 24 and 34 weeks' gestation (fasting 75g glucose load with fasting glucose level <7.8 mmol/L and 2 hour glucose level 7.8-11.0 mmol/L). Women randomised to the intervention group received dietary advice, advice about self-monitoring of blood glucose concentrations, and treatment with insulin if blood glucose concentrations were persistently elevated[141]. Women randomised to the routine care group were unaware of the results of their oral glucose tolerance test unless diagnostic of overt diabetes, as were their care providers[141]. Ongoing routine clinical care was provided as per hospital guidelines at the time[141]. The diagnosis and treatment of GDM was associated with a reduction in the risk of the primary neonatal adverse outcome (one or more of death, shoulder dystocia, bone fracture, or nerve palsy) compared with routine care (Intervention Group n=7 (1%) versus Routine Care Group n=23 (4%), adjusted relative risk 0.33 (95% CI 0.14-0.75), p=0.01), with a number needed to treat to prevent a serious adverse infant outcome of 34 (95% CI, 20-103)[141]. While diagnosis and treatment of GDM was associated with an increased chance of requiring induction of labour, there were no differences in the rate of caesarean birth[141].

Landon and colleagues conducted a similar trial in which women who had a positive oral glucose challenge test (non-fasting 50g load with 1 hour blood glucose concentration 7.5-11.1 mmol/L) underwent a fasting 3-hour 100g oral glucose tolerance test[142]. A total of 958 women were diagnosed with mild GDM (fasting glucose level of 5.3 mmol/L, and two or three of the following: 1 hour blood glucose >10.0 mmol/L, 2 hour blood glucose >8.6 mmol/L, 3 hour >7.8 mmol/L) and were randomised to receive nutritional counselling and diet therapy, with insulin if required, or usual antenatal care[142]. Diagnosis and

treatment of mild GDM was not associated with a reduction in frequency of the primary composite neonatal adverse outcome (defined as one of perinatal mortality, neonatal hypoglycaemia, hyperbilirubinaemia, neonatal hyperinsulinaemia, and birth trauma)[142]. However, diagnosis and treatment of mild GDM was associated with a reduction in mean birth weight, frequency of birth weight >4000g, frequency of LGA, shoulder dystocia and mean neonatal fat mass[142]. Women recruited and randomised to receive treatment for mild GDM were less likely to require caesarean birth, or develop preeclampsia or gestational hypertension[142]. Despite differences in inclusion criteria and diagnostic criteria[141, 142], the results of these two studies suggest that treatment of glucose intolerance less severe than overt diabetes mellitus is associated with improvements in maternal and infant outcomes.

Although hyperglycaemia seen in overt diabetes mellitus is associated with adverse maternal and infant outcomes, the degree to which less severe hyperglycaemia is associated with adverse perinatal outcomes is less clear. The Hyperglycaemia and Adverse Pregnancy Outcomes (HAPO) Study was an observational cohort study in which 25,505 participants underwent a fasting 75g oral glucose tolerance test between 24 and 32 weeks' gestation[143]. Women and caregivers were blinded to the results of the oral glucose tolerance test, unless the results were diagnostic for overt diabetes mellitus (fasting plasma glucose level >5.8 mmol/L, 2 hour plasma glucose level >11.1 mmol/L)[143]. Findings identified a strong continuous positive association, with no threshold effect, between increasing plasma glucose concentrations at fasting, 1 hour and 2 hours, and rates of primary caesarean section, birth weight >90th percentile, clinical neonatal hypoglycaemia, and cord-blood serum C-peptide[143]. While these results provide some evidence for an adverse effect of increasing hyperglycaemia there was no clear threshold at which to diagnose and treat GDM.

Multiple groups, over many years, have proposed differing screening regimes and diagnostic criteria for GDM[144-147]. These are summarised in Table 1.3. Internationally, significant variation exists with regards to whether screening is

universal or based on maternal risk factors, use of a one-step or two-step approach, and the diagnostic thresholds recommended (Table 1.3). In 2008, the International Association of Diabetes and Pregnancy Study Groups (IADPSG), proposed screening and diagnostic criteria based on the results of the HAPO study[143]. These recommended universal screening with a 2 hour 75 g oral glucose tolerance test, performed at 24-28 weeks' gestation[136]. In contrast to previous criteria, which were not derived in pregnancy, or were based solely on maternal risk of developing diabetes mellitus after pregnancy [136, 148, 149], the IADPSG recommended criteria identified women at an increased risk of adverse pregnancy outcomes[143]. The diagnostic thresholds recommended represent the plasma glucose concentrations associated with an odds ratio of 1.75 for the risk of birthweight above the 90th percentile, primary caesarean delivery, neonatal hypoglycaemia, and cord-blood serum C-peptide above the 90th percentile[143].

The IADPSG recommendations have been supported and recommended by multiple groups[150], however concern has remained about the widespread adoption of this approach. While a one-step approach to screening and diagnosis has advantages, the proposed diagnostic criteria have the potential to significantly increase the rate of diagnosed GDM creating a considerable healthcare burden. This concern has been borne out in two large randomised trials performed in the United States comparing the use of the IADPSG diagnostic criteria with the Carpenter-Coustan criteria[151, 152]. Use of the IADPSG criteria was associated with a 2-3-times higher rate of GDM diagnosis (up to 16.5%[151]) and subsequent increased healthcare utilisation. There were no significant differences in perinatal adverse outcomes or risk of birth of an infant of high birth weight or LGA[151, 152], suggesting that the IADPSG recommendations may not be cost-effective in routine practice.

Table 1.4 – Screening and diagnostic criteria for GDM

Organisation	Screening population	Glucose load	Fasting plasma glucose (mmol/L)	1-hour plasma glucose (mmol/L)	2-hour plasma glucose (mmol/L)	3-hour plasma glucose (mmol/L)
WHO 2013[137] One-step approach	Universal screening	75 g fasting OGTT	5.1-6.9	≥10.0	8.5-11.0	n/a
ACOG[145] Two-step approach	Universal screening	1. 50 g non-fasting OGCT 2. 100 g fasting OGTT	1. n/a 2. ≥5.3	1. ≥7.2-7.8 2. 10.0	1. n/a 2. ≥8.6	1. n/a 2. ≥7.8
Canadian Diabetes Association[153] Two-step approach	Universal screening	1. 50 g non-fasting OGCT 2. 75 g fasting OGTT	1. n/a 2. ≥5.3	1. ≥ 7.8 2. ≥10.6	1. n/a 2. ≥9.0	1. n/a 2. n/a
NICE[146] One-step approach	Risk factor based	75 g fasting OGTT	≥5.6	n/a	≥7.8	n/a
IADPSG[136] One-step approach	Universal screening	75 g fasting OGTT	≥5.1	≥10.0	8.5-11.0	n/a
ADIPS[150] One-step approach	Universal screening	75 g fasting OGTT	5.1-6.9	≥10.0	8.5-11.0	n/a

WHO, World Health Organisation. ACOG, American College of Obstetricians and Gynecologists. NICE, National Institute for Health and Care Excellence. IADPSG, International Association of the Diabetes and Pregnancy Study Groups. ADIPS, Australian Diabetes in Pregnancy Society.

1.5.1.1 Maternal, infant and childhood risks associated with gestational diabetes

While diagnostic criteria may vary, women diagnosed with GDM are at increased risk of developing hypertensive disorders of pregnancy, requiring induction of labour, perineal trauma, and birth by caesarean section (Table 1.4)[154-156]. Infants born to women who have GDM are at increased risk of being born preterm[154, 157], being born with high birth weight, or LGA[154-156], and birth trauma, including shoulder dystocia (table 1.5)[155, 156]. Infants born to women with GDM are also more likely to be admitted to the neonatal intensive care unit, predominantly because of respiratory distress requiring support[154], or hypoglycaemia requiring invasive treatment[154, 157].

The future risk for children born to women with GDM remains uncertain. While some suggest an increased risk of childhood and adolescent obesity and impaired glucose tolerance[158-160], others do not[124, 161]. It is possible that maternal overweight and obesity plays a role in confounding the relationship between GDM and childhood and adolescent obesity[124, 162]. Furthermore maternal overweight and obesity may represent a stronger effect on these longer term risks[124].

Table 1.5 – Risk of adverse maternal outcomes in women with GDM compared to women without GDM

Outcome	Estimate of increased risk	References
Hypertensive disorders of pregnancy	1.7	(154, 157)
Induction of labour	1.5	(157)
Perineal trauma	1.5	(157)
Caesarean section	1.8	(154, 157, 163)

Table 1.6 – Risk of adverse neonatal outcomes in women with GDM compared to women without GDM

Outcome	Estimate of increased risk	References
Preterm delivery	1.7	(154, 157)
LGA/macrosomia	4.6	(154, 157, 163, 164)
NICU admission	4.0	(154)
Respiratory distress	2.0	(154)
Hypoglycaemia	3.4	(154, 157)

1.5.2 Mechanisms for fetal growth in maternal overweight and obesity

The mechanisms underlying the effect of maternal overweight and obesity on fetal growth are complex. A significant contribution is likely via increased insulin resistance, hyperglycaemia and hyperinsulinaemia, as outlined in the Pedersen hypothesis[134]. Women who are overweight or obese in pregnancy have been shown to have higher serum glucose concentrations than women with a normal BMI, even below the diagnostic threshold for a diagnosis of gestational diabetes[165]. However, maternal hyperglycaemia and hyperinsulinaemia do not explain the whole effect of maternal overweight and obesity on fetal growth[66]. Women who are overweight or obese, and have well-controlled GDM, remain twice as likely to deliver a macrosomic or LGA infant, as compared with women of a normal BMI[166]. In the HAPO study, maternal overweight and obesity were associated with an increased risk of birth of a macrosomic infant, even when controlling for maternal glucose concentrations and in the absence of GDM[167, 168].

Another likely contributing factor to fetal growth may lie in maternal adipose tissue, which is metabolically active. Adipose cells and macrophages resident in adipose tissue produce cytokines such as TNF-**α** and IL-6, which contribute to a state of chronic low grade inflammation among people who are overweight or obese[169, 170]. This low grade inflammatory state is thought to contribute to increased insulin resistance[169]. In pregnancy, TNF- **α** and IL-6 may contribute to increased placental nutrient transfer, thereby facilitating increased fetal growth[171]. However, evidence is conflicting as to whether women who are overweight or obese have elevated concentrations of inflammatory cytokines in pregnancy[172-176], and whether these inflammatory cytokines are associated with an increased risk of delivering an infant LGA[172, 177].

Another potential mechanism contributing to fetal growth among women who are overweight or obese is that of maternal lipids. It is hypothesised that maternal circulating triglycerides are converted to free fatty acids in the placenta and then transported into the fetal circulation, where they contribute to fetal fat

deposition[66, 178]. In keeping with this this hypothesis, both maternal triglyceride concentrations and free fatty acid concentrations have been associated with increased risk of delivering an infant LGA[179-181]. Women who are overweight or obese in early pregnancy have been shown to have higher concentrations of triglycerides and VLDL cholesterol, and lower concentrations of HDL cholesterol in the third trimester[177]. While the external validity of these findings is limited as a result of small and highly select patient samples, they suggest that the underlying mechanisms involved in fetal growth among women who are overweight or obese are multifactorial and complex, requiring further elucidation.

1.5.3 Effect of maternal overweight and obesity on fetal growth trajectories

Published work to date on fetal growth trajectories has focused on women with a normal BMI. However, it cannot be assumed that the fetuses of women who are overweight or obese have similar growth trajectories to the fetuses of women with a normal BMI. Further data investigating the effect of maternal BMI on fetal growth trajectories is required.

In a prospective cohort of over 4000 women, Sovio and colleagues[182] identified fetal abdominal circumference measures among obese women were more likely to be greater than the 90th percentile, an effect evident from 20 weeks' gestation. Maternal obesity was also identified as an independent predictor of abdominal circumference greater than the 90th percentile from as early as 24 weeks' gestation among women with GDM or impaired glucose tolerance[183].

Ay and colleagues[184] performed a large prospective cohort study of over 8000 women in the Netherlands, to evaluate the effect of maternal BMI on estimated fetal weight z-scores throughout pregnancy. Maternal BMI was self-reported (average 23.6 kg/m^2)[184]. Fetuses of women in the highest BMI quintile had significantly higher estimated fetal weight z-scores from 20 weeks' gestation[184]. Furthermore, prepregnancy BMI was associated with an increase in fetal growth rate over the second and third trimesters[184].

The US National Institute of Child Health and Human Development Fetal Growth Studies investigated the effect of maternal obesity on all fetal biometry trajectories throughout pregnancy[185]. A total of 2,763 women were prospectively enrolled in early pregnancy, of whom 443 had a BMI ≥30 kg/m^2. Findings indicate that estimated fetal weight was, on average, 30gm greater in obese women compared with normal-weight and overweight women, an effect evident from 30 weeks' gestation. Furthermore, this difference in weight increased over the third trimester, reaching almost 100gm by 38 weeks' gestation[185].

In considering individual fetal biometry measures, Zhang and colleagues demonstrated that maternal obesity was associated with longer length of the femur and humerus from 21 weeks' gestation, as well as greater head circumference measurements in the third trimester[185]. While this is a contemporary population of women, it is not entirely applicable to an Australian population. The cohort included a large proportion of Hispanic women, and the actual number of obese women recruited was relatively small (468 women, 16%). Maternal obesity was considered as a categorical variable[185], further limiting the statistical power to detect differences.

Dodd and colleagues have published fetal biometry and estimated fetal weight trajectories for women prospectively recruited to the LIMIT randomised trial[118]. This involved a contemporary Australian cohort of almost 2000 women who were overweight or obese in early pregnancy. The average BMI in this cohort was 31.2 kg/m^2, with 60% of women having a BMI ≥30 kg/m^2. All fetal biometry and estimated fetal weight z-scores were greater than population means[118]. However, in contrast to the findings of Zhang and colleagues[185], these differences did not increase in the third trimester, instead following currently used population fetal growth curves[118]. How these findings compare to a contemporary cohort of women with a normal BMI is unclear.

Taken together, this evidence suggests the fetuses of women who are overweight or obese may both be significantly larger at any given gestational age, and may have increased growth trajectories over pregnancy. However, when, over pregnancy, fetal growth diverges, is not known.

1.5.4 Interactions of maternal overweight and obesity and gestational diabetes on fetal/neonatal growth

An emerging clinical question is the relative contribution of maternal overweight and obesity and GDM on fetal and neonatal growth. Maternal overweight and obesity and GDM commonly coexist. They have a common metabolic milieu, consisting of hyperglycaemia, hyperinsulinaemia and insulin resistance [168], although the relative contributions and additive effects of each is difficult to determine.

Catalano and colleagues re-analysed the HAPO data [168] to evaluate the relative contributions of maternal BMI and GDM on adverse pregnancy outcomes. Women were grouped into one of four groups– no GDM + no obesity; GDM + no obesity; no GDM + obesity; GDM + obesity. GDM was defined according to the IADPSG criteria[136]. In this analysis both GDM alone and obesity alone were associated with an increased risk of LGA, higher newborn percentage body fat, cord blood C peptide >90th percentile, primary caesarean section and pre-eclampsia, when compared with women of a normal BMI and without GDM[168]. For women who were both obese and were diagnosed with GDM, the risk for all adverse pregnancy outcomes was greater than either alone, suggesting an additive effect[168]. Strengths of this work include the prospective observational nature of the HAPO study, and the large numbers of women recruited. These findings are consistent with other reports in the literature, where women with both obesity and GDM, were identified to have higher rates of LGA infants compared with women who had GDM and were not obese[186]. This study, however, is limited by its retrospective nature, the diagnosis and testing for GDM undertaken based on clinical risks factors rather than universal screening, and the use of different diagnostic thresholds to those currently recommended[186].

Furthermore, maternal BMI was determined at 28 weeks' gestation, rather than measured prior to or in early pregnancy. This is also a secondary exploratory analysis and results require cautious interpretation.

It has been suggested that maternal overweight and obesity may have a greater effect on fetal and offspring growth than a diagnosis of GDM. A retrospective study of 9,835 women estimated that maternal overweight and obesity was associated with a population attributable risk of approximately 20% for LGA, whereas GDM among women who were not overweight or obese contributed approximately 3% of LGA infants [187]. However, the risk remained highest among women who were both overweight or obese and who were diagnosed with GDM, contributing approximately 24% of LGA infants[187]. While this study was large, there was a high proportion of Hispanic women, and it is yet to be seen whether these results are relevant to different ethnic populations.

A large prospective ultrasound study in the UK obtained fetal abdominal circumference measures and growth in a cohort of 4,069 women[182]. Women who were obese were more likely to have a fetus with an abdominal circumference measurement >90th percentile at the 20 week morphology scan, a finding that persisted throughout the third trimester[182]. Among obese women who developed GDM, there was a five times increased risk of the fetus having an abdominal circumference >90th percentile, greater than the effect of either maternal obesity or GDM alone[182]. Abdominal circumference alone is a crude measure of fetal adiposity. The presence of fetal overgrowth as early as the routine 20 week ultrasound among obese women, and not those who go on to develop GDM, suggests that the effects of maternal overweight and obesity on fetal growth begin in very early pregnancy, and potentially are mediated through other pathways in addition to hyperglycaemia.

1.6 Antenatal interventions to impact fetal growth and adiposity and prevent LGA

1.6.1 Diet and lifestyle interventions to improve perinatal outcomes in women who are overweight and obese

There have been multiple studies investigating the effects of antenatal diet and lifestyle interventions on pregnancy outcomes in women who are overweight or obese. While there is significant variation with regards to the intensity and type of diet and lifestyle interventions investigated, overall, they demonstrate only a modest reduction in gestational weight gain with little or no beneficial effect on infant outcomes including high birth weight.

The two largest randomised trials investigating the effect of antenatal diet and lifestyle interventions on pregnancy outcomes in obese women are the LIMIT randomised trial [188] and the UPBEAT Study[189]. The LIMIT study was conducted in Adelaide, South Australia [188], and is the largest single randomised controlled trial evaluating antenatal diet and lifestyle interventions to improve pregnancy outcomes in women who were overweight or obese. The intervention was a combination of dietary, exercise and behavioural strategies delivered via face-to-face meetings and phone conversations. A total of 2,122 women were recruited[188]. Women randomised to the diet and lifestyle intervention were successful in modifying diet and physical activity over the course of the trial[190], however there was no evidence of an effect on clinical outcomes[188]. There was no significant difference in overall average gestational weight gain between the Lifestyle Advice and Standard Care groups [188].

The UPBEAT study was a multicentre randomised controlled trial recruiting 1,555 women with a BMI ≥30 kg/m^2 and a singleton pregnancy[189]. The diet and lifestyle intervention was relatively intensive, with weekly group or individual sessions consisting of advice on behaviour change and provision of information about healthy patterns of eating [189]. The physical activity component of the intervention focused on incremental increases in walking at moderate intensity [189]. While women improved their dietary intake and increased physical activity, this did not impact the risk of GDM, or high infant birth weight[189], with only a modest effect on mean gestational weight gain (mean Standard Care 7.76 kg (SD

4.6 kg), mean Intervention Group 7.19 kg (SD 4.6 kg), estimated mean difference -0.55kg (95% CI, -1.08, -0.02), p=0.041)[189].

The International Weight Management in Pregnancy (i-WiP) collaborative group conducted an individual patient data meta-analysis of randomised trials assessing the effect of diet and physical activity interventions on pregnancy outcomes. A total of 36 studies and data from over 12,000 women were included[191]. While diet- and physical-activity-based interventions had a modest impact in reducing gestational weight gain (mean difference -0.70 kg, 95% CI -0.92 to -0.48 kg) there was no significant difference in adverse maternal or fetal/neonatal outcome[191]. While studies including women across the BMI range were included, secondary analysis indicated that the effect of the intervention did not significantly vary by maternal BMI[191]. In an associated cost-effectiveness analysis diet- and physical-activity-based interventions in pregnancy were not cost-effective compared with usual care [191].

With consideration to the variation in the type and intensity of intervention, and that some individual studies have shown some inconsistent differences in maternal and infant outcomes, strong enough evidence does not exist to support recommending antenatal diet and lifestyle interventions in routine clinical practice. Consideration of alternative antenatal interventions to improve outcomes among women who are overweight or obese is required.

1.6.2 Metformin to improve perinatal outcomes in women who are overweight and obese

Metformin is an oral biguanide medication that increases peripheral insulin sensitivity and decreases hepatic glucose production. It is commonly used in the treatment of type 2 diabetes mellitus. The safety and efficacy of metformin, in comparison with insulin, for the treatment of GDM, was demonstrated in a large Australian randomised, open-label trial – the Metformin in Gestational Diabetes Trial (MiG Trial)[192]. Maternal overweight and obesity and diabetes mellitus (both pre-existing type 2 diabetes mellitus and GDM) commonly coexist and

have a common metabolic milieu, consisting of hyperglycaemia, hyperinsulinaemia and insulin resistance[168]. This has led to a number of researchers evaluating the antenatal use of metformin in women who are overweight or obese without pre-existing diabetes mellitus[193-196].

Antenatal metformin alone, as an intervention to improve pregnancy outcomes among women who are overweight or obese in early pregnancy, has been assessed in one randomised trial to date[194]. The EMPOWaR randomised trial recruited and randomised 449 women with an early pregnancy BMI ≥30 kg/m^2 to receive either antenatal metformin (up to a maximum of 2500mg per day) or matching placebo[194]. Chiswick and colleagues reported no significant differences in total gestational weight gain or other maternal outcomes, including risk of gestational diabetes[194]. They also did not find any significant differences in infant birthweight or neonatal outcomes[194].

Antenatal metformin as an adjuvant to diet and lifestyle advice has been assessed in three randomised trials[193, 195, 196]. The GRoW randomised trial recruited and randomised 524 women with an early pregnancy BMI ≥25.0 kg/m^2 to receive either antenatal metformin (up to 2000mg daily) or matching placebo[193]. All women recruited to the trial received an antenatal diet and lifestyle intervention[193]. While women randomised to receive adjuvant metformin had lower average weekly gestational weight gain (adjusted mean difference -0.08 kg, 95% CI -0.14 to -0.02, p=0.007), and were more likely to have gestational weight gain below recommendations (aRR 1.46, 95% CI 1.10 to 1.94), total gestational weight gain did not differ significantly [193]. There were no significant differences in other maternal or infant outcomes[193].

In a second randomised trial, Syngelaki and colleagues recruited and randomised 450 women with an early pregnancy BMI ≥35 kg/m^2 to receive either antenatal metformin (up to a maximum of 3000mg per day) or matching placebo, commencing prior to 20 weeks' gestation[195]. While there was a small difference in total gestational weight gain over the trial (metformin vs placebo), infant birthweight and risk of delivering an infant LGA were not significantly

different[195]. Interestingly, risk of developing preeclampsia was significantly lower among women who were randomised to receive antenatal metformin in this trial (Antenatal Metformin 3.0% vs Placebo 11.3%, OR 0.24, 95% CI 0.10-0.61, p=0.001)[195], however this was an exploratory secondary outcome, and has not been consistently shown.

In the third randomised trial, Nascimento and colleagues included women with an early pregnancy BMI ≥30 kg/m^2, in which women were randomised to receive either low dose metformin (up to maximum 1000mg per day) or no metformin (Control group)[196]. All women received a diet and lifestyle intervention focused on reducing calorie intake and increasing physical activity[196-198]. There were no significant differences between the metformin and control group with regards to gestational weight gain or risk of gestational diabetes[197, 198]. Women randomised to the metformin group were less likely to have a caesarean birth (Metformin 39.8% vs Control 62.9%, p<0.01)[196], however the very high rate of caesarean birth among women randomised to the Control group (62.9%) likely limits the generalisability of these findings. Information regarding prior mode of delivery and gestational age at delivery were also missing[196]. Women randomised to receive adjuvant antenatal metformin were also less likely to be diagnosed with pre-eclampsia (Metformin 3.5% vs Control 4.8%, p<0.01)[196]. This was a secondary exploratory outcome, and represented only a very small absolute risk reduction.

In summary, antenatal metformin, either alone or as an adjuvant to diet and lifestyle advice, is not associated with significant differences in maternal or infant outcomes, among women who are overweight or obese in early pregnancy. While small differences in gestational weight gain have been shown in some trials[193, 195] this finding is not consistent, and is not associated with improvement in perinatal outcomes. The effects of antenatal metformin, alone or as adjuvant to antenatal diet and lifestyle advice, on fetal growth and adiposity, are not known. The women in all three trials were recruited and commenced metformin between 10 and 20 weeks' gestation. It is therefore possible that

interventions to improve pregnancy outcomes may require commencement earlier in pregnancy, or prior to pregnancy, to be effective.

1.6.3 Effect of antenatal interventions on fetal growth and growth trajectories

There is little data comparing the effects of antenatal diet and lifestyle interventions, and/or antenatal metformin on fetal growth among women who are overweight or obese. While the evidence suggests antenatal diet and lifestyle interventions have no significant effect on risk of infant macrosomia and LGA, little is known about intrauterine fetal growth and growth trajectories among women exposed to an antenatal diet and lifestyle interventions, alone or in conjunction with oral metformin.

A secondary analysis of fetal ultrasound data from the LIMIT randomised trial [199] investigated the effect of an antenatal diet and lifestyle intervention on fetal growth and adiposity [118]. Women participated in a research ultrasound at 28 and 36 weeks' gestation, during which routine fetal biometry, amniotic fluid index (AFI) and Doppler measurements were obtained, an estimated fetal weight calculated and ultrasound measures of fetal adiposity performed in a standardised manner[118]. Of the 2,212 women who participated in the LIMIT trial, 1,847 women had a research ultrasound at one or both time points[118]. Although there were no differences in fetal biometry measures identified, fetuses of women randomised to the Lifestyle Intervention had greater mid-thigh fat mass, and a slower rate of subscapular fat mass accumulation[118]. The authors hypothesised that this differential location of fetal fat may represent a more favourable phenotype[118]. Whether this effect differs among women of a normal BMI is uncertain, as is the the effect of a combined antenatal diet and lifestyle intervention and metformin.

1.7 Research gaps identified

- What is the effect of maternal BMI, across the BMI spectrum, on
    - Fetal biometry measures and estimated fetal weight?

    - Fetal adiposity measures and fetal body composition?
    - The mediating effect of a diagnosis and treatment of GDM on fetal biometry and adiposity measures?
- Do infants born LGA, compared with those not born LGA, exhibit differences in
    - Fetal biometry measures and fetal biometry measure trajectories?
    - Fetal adiposity measures and fetal adiposity measure trajectories?
    - When during pregnancy these differences become evident?
- What effect does adjuvant antenatal metformin, with a diet and lifestyle intervention, have on fetal body composition among women who are overweight or obese in early pregnancy?
- What effect does an antenatal diet and lifestyle intervention have on fetal body composition among women who have a normal BMI in early pregnancy?

Chapter 2: Methods

2.1 The clinical cohort

This thesis examines the effect of maternal BMI on fetal growth and adiposity, antenatal contributors to fetal growth and adiposity, and the effect of antenatal interventions on fetal growth and adiposity using data from a set of three harmonised randomised controlled trials conducted between June 2008 and April 2017. Study protocols were purposefully designed ensuring the studies were sufficiently similar to allow for valid comparisons between them, and for the data from the studies to be combined.

The three randomised trials recruited women with a singleton pregnancy, between 10^{+0} and 20^{+0} weeks' gestation, who received pregnancy care and birthed at one of three major maternity hospitals in Adelaide, South Australia – the Women's and Children's Hospital, Flinders Medical Centre, or the Lyell McEwin Hospital. Women were excluded if they had a multiple pregnancy or type 1 or 2 diabetes mellitus diagnosed prior to pregnancy. The antenatal interventions in all trials were additional to standard antenatal care, provided according to local hospital and state pregnancy care guidelines[200]. Randomisation for all three trials was performed using computer-generated schedules, with stratification for maternal parity (0 versus ≥1)[188, 193, 201], and (for the LIMIT and GRoW randomised trials) additionally by BMI at antenatal booking (25.0-29.9 versus ≥30.0 kg/m^2), and collaborating centre[188, 193].

During the recruitment period, guidelines for routine pregnancy care remained consistent[200], with the exception of screening and diagnosis of gestational diabetes[202]. Prior to 2015, the diagnostic criterion for gestational diabetes was a positive 75gm oral glucose tolerance test at 28 weeks' gestation with fasting blood glucose >5.5 mmol/L or 2hr ≥7.8 mmol/L[202]. From 2015, the Australian Diabetes in Pregnancy Society (ADIPS) recommendations were adopted, where women were diagnosed with gestational diabetes based on a 75gm oral glucose tolerance test at 28 weeks' gestation with one or more of the following: fasting

blood glucose ≥5.1 mmol/L, 1hr ≥10.0 mmol/L, or 2hr ≥8.5 mmol/L[202, 203]. This change in diagnostic criteria impacted women recruited to the GRoW and Optimise randomised trials (see Table 2.1). Following diagnosis, women were provided with dietary advice and were encouraged to perform home blood glucose monitoring four times daily [before breakfast (Fasting) and 2 hours after the start of each meal (postprandial)], with fasting blood glucose measurements targeted between 3.5 and 5.5 mmol/L, and postprandial blood glucose measurements targeted between 4.0 and 7.0 mmol/L[202]. Medical treatment with insulin or metformin was considered if fasting blood glucose measurements were ≥ 5.5 mmol/L once or more per week and/or if postprandial blood glucose measurements were ≥7.5 mmol/L twice or more per week[202]. Advice regarding gestational weight gain was not a part of routine pregnancy care, irrespective of early pregnancy maternal BMI.

In all three trials, women were invited to attend for a research ultrasound at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks of gestation, with fetal biometry and adiposity measures obtained as described below. All research ultrasounds were performed by a medical practitioner with specialist or subspecialist training in obstetric ultrasound, while blinded to the participant's allocated treatment group. A small number of women who underwent a clinical ultrasound during these gestational windows consented to the inclusion of their fetal biometry measurements but did not contribute fetal adiposity measures.

Table 2.1 - Details of the trials from which fetal ultrasound data was obtained

	LIMIT[188, 190]	GRoW[204]	Optimise[201]
Maternity centre	WCH, FMC, LMH	WCH, LMH	WCH
Maternal BMI (Kg/m^2)	≥25.0	≥25.0	18.5-24.9
Antenatal intervention and comparator	Diet and lifestyle vs. Standard care	Metformin vs. placebo (All women received diet and lifestyle intervention)	Diet and lifestyle vs. Standard care
Sample size (N)	2212	524	645
Primary outcome	Infant large for gestational age (> 90th percentile for gestational age and gender)	Infant birthweight >4000 gm	Infant birthweight >4000 gm
Principal findings	No reduction in risk of infant LGA (19% vs. 21%). Significant reduction in risk of birthweight >4000 gm (15% vs. 19%). No significant difference in mean total gestational weight gain. Increased daily fruit, vegetable, and fibre intake, and reduced saturated fats. Increased maternal physical activity.	No significant reduction in risk of infant birthweight >4000 gm. Lower average weekly gestational weight, and more likely to have gestational weight gain below recommendations, however no significant difference in total gestational weight gain. No significant difference in maternal diet quality or physical activity measures.	No significant reduction in risk of infant birthweight >4000 gm. Significant improvement in dietary quality. No significant difference in reported physical activity. No significant difference in total gestational weight gain.

WCH, Women's and Children's Hospital. FMC, Flinders Medical Centre. LMH, Lyell McEwin Hospital.

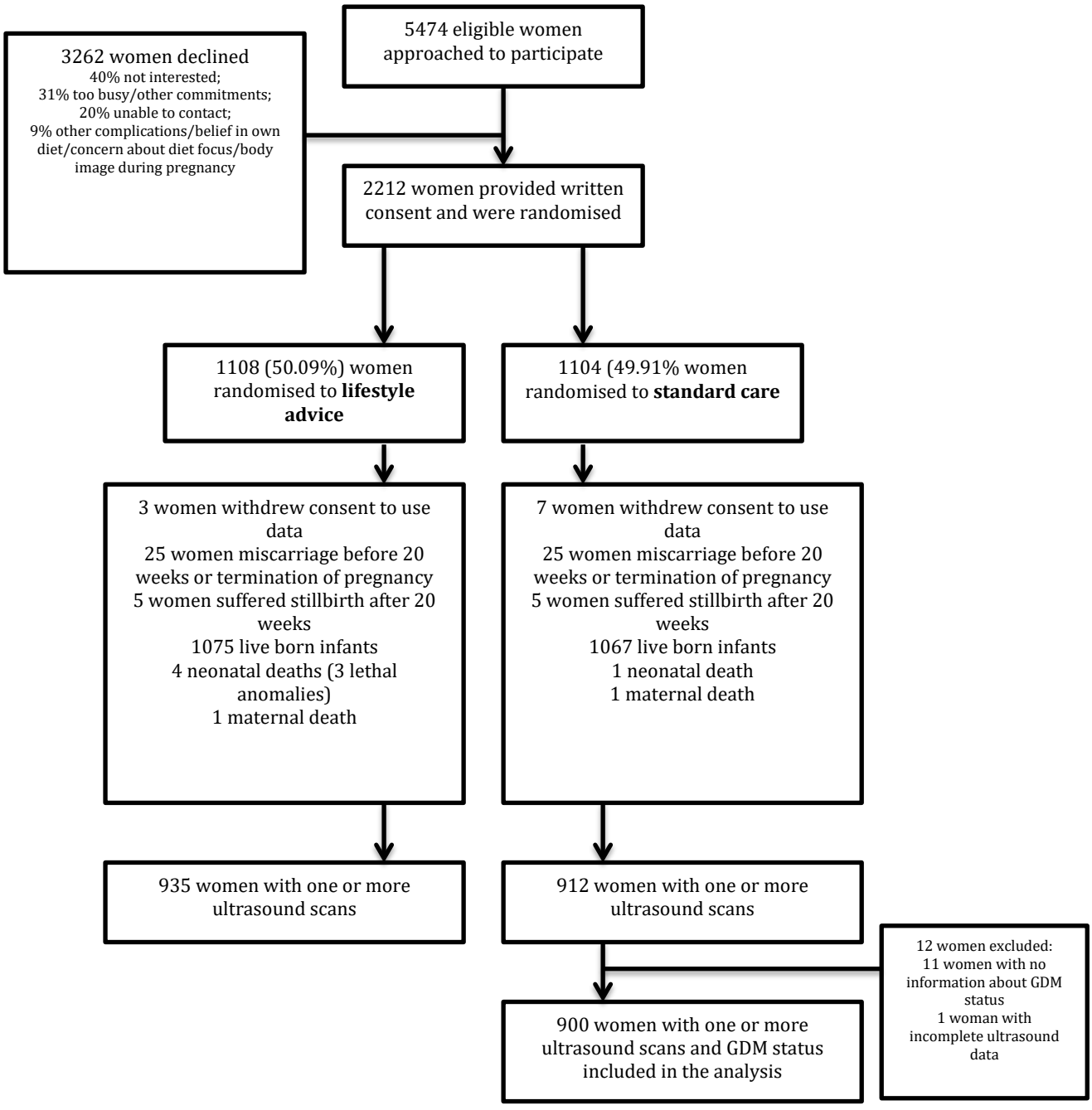
2.2 Antenatal dietary and lifestyle intervention and control

Women randomised to the Lifestyle Advice group of the LIMIT and Optimise randomised trials[188, 201], and all women recruited to the GRoW randomised trial[193] received a comprehensive, tailored dietary and lifestyle intervention over the course of their pregnancy, administered by a research dietitian and trained research assistants. Dietary advice was in keeping with current Australian standards, and included maintaining a balance of carbohydrates, fat, and protein intake; reducing intake of foods high in refined carbohydrates and saturated fats; increasing fibre intake; and aiming for intake of two serves of fruit, five serves of vegetables, and three serves of dairy per day[205]. Physical activity advice focused mainly on increasing daily walking and incidental activity. The intervention was provided over multiple sessions, both in person and over the phone. Following an initial planning session, and a follow up session with a research dietitian at 28 weeks' gestation, additional contact with the research assistants occurred at 22, 24, 32, and 36 weeks' gestation. Women were encouraged to set achievable goals, identify barriers to change, and provided with support to problem solve and make dietary and lifestyle changes over the course of the pregnancy.

2.3 The LIMIT randomised trial

The LIMIT trial recruited women with a BMI ≥25 kg/m^2 (measured in early pregnancy) and who had a singleton pregnancy between 10^{+0} and 20^{+0} weeks of gestation. In total, 2212 women were randomised (Lifestyle Advice Group [n=1108] or Standard Care Group [n=1104]) (Figure 2.1) using a computer-generated schedule, with stratification for maternal parity (0 versus ≥1), BMI at antenatal booking (25.0-29.9 versus ≥30.0 kg/m^2), and collaborating centre. Of these, 1,847 women (935 Lifestyle Advice, 912 Standard Care) underwent one or more research ultrasounds over pregnancy (Figure 2.1).

Figure 2.1. Participant flow: women included in the analysis of ultrasound measures of fetal growth and adiposity in the LIMIT randomised trial



2.3.1 The LIMIT randomised trial: outcomes and primary findings

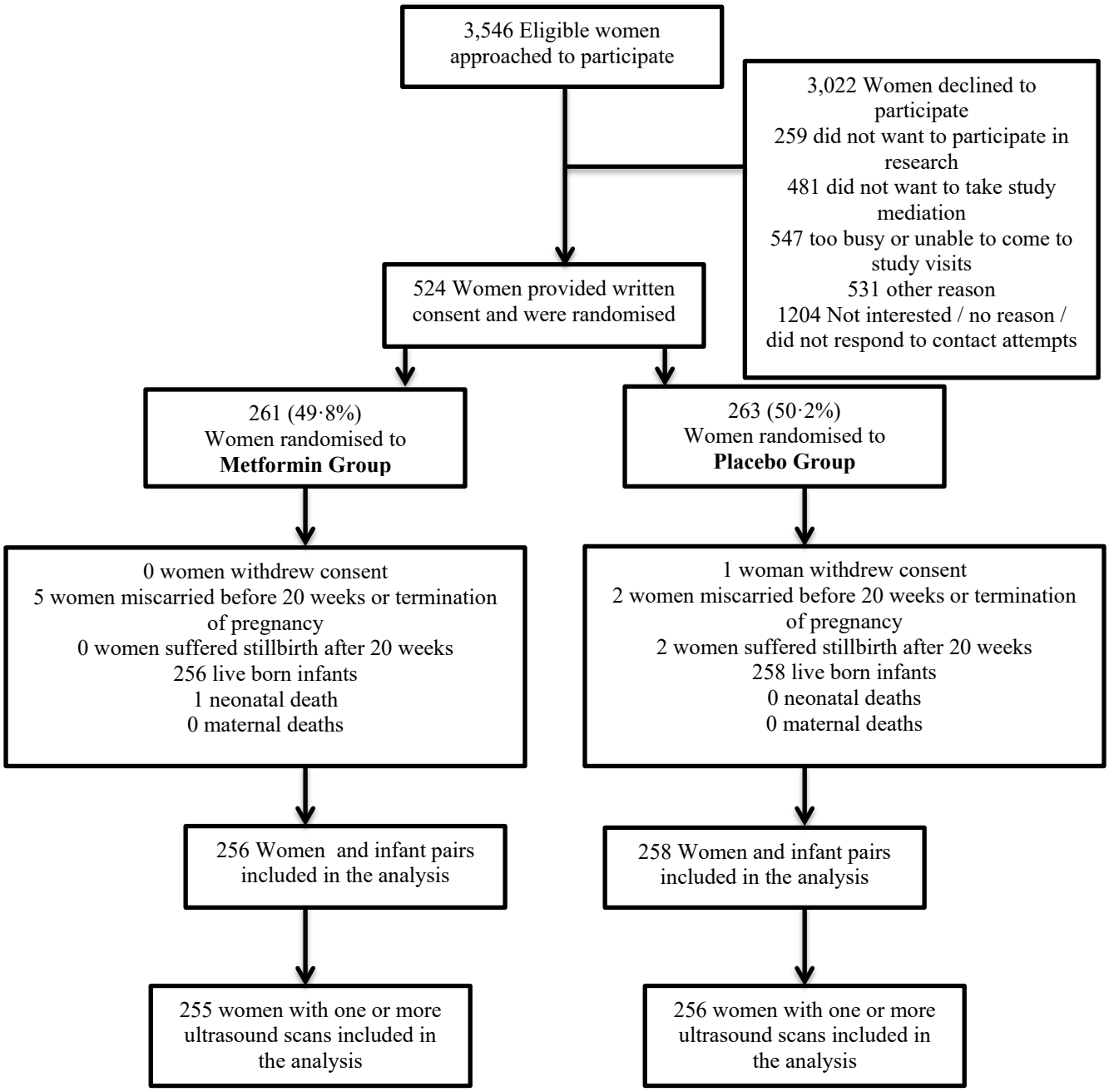
A summary of women primary outcomes and principal findings for women recruited to the LIMIT randomised trial are detailed in Table 2.1. The LIMIT randomised trial had a primary outcome of infant large for gestational age (birthweight >90th percentile for gestational age and infant sex)[188]. While no statistically significant difference in occurrence of infants born LGA was identified (203/1075 (19%) Lifestyle Advice group vs. 224/1067 (21%) Standard Care group; adjusted relative risk 0.90, 95% confidence interval 0.77, 1.07; p=0.24), provision of the dietary and lifestyle intervention was associated with a statistically significant difference in risk of delivering an infant with birthweight >4000gm (164/1075 (15%) Lifestyle Advice group vs. 201/1067 (19%) Standard Care group; adjusted relative risk 0.82; 95% confidence interval 0.68, 0.99; p=0.04)[188]. Women in the Lifestyle Advice group successfully increased their intake of fruit and vegetables, dietary fibre and reduced intake of saturated fats[190]. Women randomised to the Lifestyle Advice group also reported greater total physical activity than women randomised to the Standard Care group[190].

2.4 The GRoW randomised trial

The GRoW randomised trial recruited women with a BMI ≥25 kg/m^2 (measured in early pregnancy) and a singleton pregnancy between 10^{+0} and 20^{+0} weeks of gestation[204, 206]. In total, 524 women were recruited and randomised to this study (Figure 2.2). Women were stratified according to parity (0 versus ≥1), BMI at booking visit (25.0-30.0 kg/m^2 versus ≥30.0 kg/m^2), and collaborating centre. A total of 10 women withdrew, underwent a termination of pregnancy, or suffered a miscarriage before 20 weeks' gestation, resulting in 256 women and their infants in the Metformin Group and 258 women and their infants in the Placebo group who were included in primary analyses[193] (Figure 2.2). A total of 511 women attended for one or more research ultrasound over the course of their pregnancy, 255 women from the Metformin Group and 256 women from

the Placebo Group (Figure 2.2). All women recruited to the GRoW randomised trial received the antenatal dietary and lifestyle intervention as described above for the LIMIT trial.

Figure 2.2: Participant flow: women included in the analysis of ultrasound measures of fetal growth and adiposity in the GRoW randomised trial



2.4.1 The GRoW randomised trial: Intervention and control

In addition to the dietary and lifestyle advice, women recruited to the Metformin Group received a supply of oral metformin tablets (500mg) and were instructed to start taking one tablet per day for the first week, then increase to a maximum of two tablets twice daily (maximum 2000mg daily) over four weeks as tolerated, and to continue over the course of the pregnancy. Women allocated to the Placebo Group received a supply of placebo tablets identical in taste and appearance to the metformin tablets, and similarly instructed to start taking one tablet per day for the first week, then increase to a maximum of two tablets twice daily over four weeks as tolerated and continue over the course of the pregnancy. The metformin and placebo tablets were packaged by an independent pharmaceutical packaging company (Pharmaceutical Packaging Professionals, Victoria).

2.4.2 The GRoW randomised trial: Outcomes and primary findings

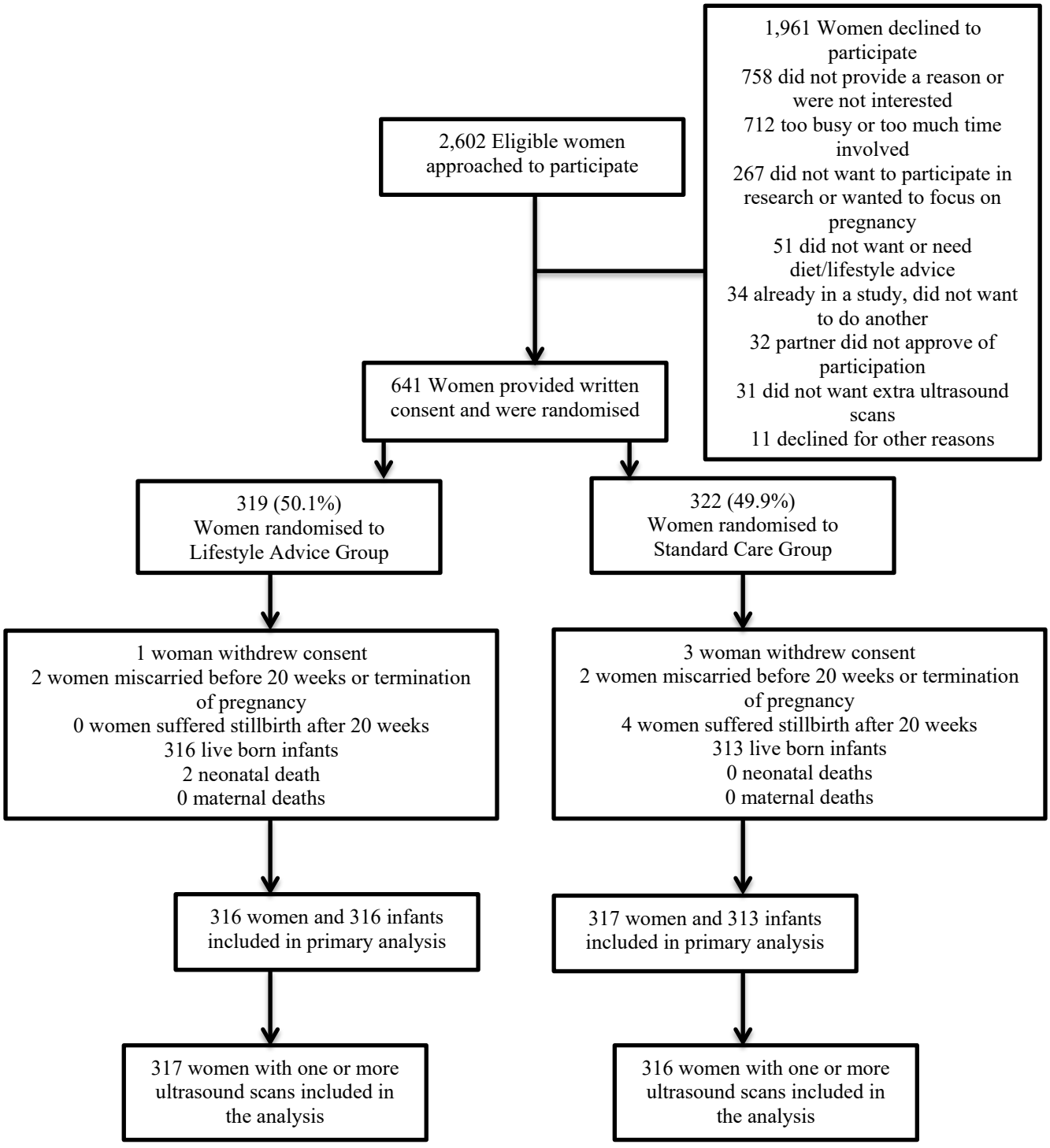
A summary of women recruited to the GRoW randomised trial, the primary outcome and principal findings are shown in table 2.1. The primary outcome of the GRoW randomised trial was delivery of an infant with birthweight >4000 g[204]. No statistically significant effect of adjuvant metformin on birthweight >4000g was identified (Metformin group 40/256 [15.63%] versus Placebo group 37/258 [14.34%]; adjusted treatment effect 0.97; 95% CI 0.65 to 1.47; p = 0.899)[204]. While antenatal treatment with oral metformin was associated with a reduction in average weekly gestational weight gain, this difference was small and unlikely of clinical relevance, particularly as there was no difference in total gestational weight gain[204]. There were no statistically significant differences in other secondary maternal or infant outcomes[204].

2.5 The Optimise randomised trial

The Optimise randomised trial recruited women who had a normal BMI (18.5-24.9 kg/m^2) at their first antenatal booking visit, and were recruited only from

WCH [201, 207]. As for the LIMIT and GRoW trials, women could be included if they had a singleton pregnancy between 10^{+0} and 20^{+0} weeks' gestation and did not have diabetes or other health problem precluding dietary change. Randomisation was stratified by parity (0 versus ≥1). A total of 641 women were recruited and randomised to the Optimise trial (319 women Lifestyle Advice group, 322 women Standard Care group). A total of 4 women withdrew consent, 4 women suffered a miscarriage or underwent a termination of pregnancy prior to 20 weeks' gestation, and 4 women suffered a stillbirth after 20 weeks' gestation (Figure 2.3). In total, 633 women and their infants (316 women Lifestyle Advice group and 317 women Standard Care group) were included in the primary analysis[201]. All 633 women underwent at least one research ultrasound over the course of their pregnancy, and contributed clinical and ultrasound data to the analyses (Figure 2.3).

Figure 2.3: Participant flow: women included in the analysis of ultrasound measures of fetal growth and adiposity in the Optimise randomised trial



2.5.1 The Optimise randomised trial: Intervention and control

In total, 641 women were randomised to either Lifestyle Advice (n=319) or Standard Care (n=322)[201]. The Lifestyle Advice group received the same antenatal dietary and lifestyle advice as for the LIMIT trial, described above[199]. Women randomised to the Standard Care group were managed per current state-based and local hospital guidelines[200].

2.5.2 The Optimise randomised trial: Outcomes and primary findings

Details about the women recruited to the Optimise trial, the primary study outcome, and principal findings are shown in Table 2.1. The primary outcome of the Optimise randomised trial was delivery of an infant with birthweight >4000 g[201, 208]. There was no statistically significant difference in the proportion of infants born with birthweight >4000g between the Lifestyle Advice and Standard Care groups (24/316 (7.59%) versus 26/313 (8.31%) respectively; adjusted relative risk (aRR) 0.91; 95% confidence interval (CI) 0.54, 1.55; p = 0.732)[201]. Women randomised to the Lifestyle Advice group reported improvements in their dietary quality over pregnancy, compared with women who were randomised to Standard Care[201]. However, there were no statistically significant differences for total gestational weight gain (11.32 ± 3.96 kg Lifestyle Advice versus 11.70 ± 3.78 kg Standard Care; adjusted mean difference (aMD) -0.37; 95% CI -0.97 to 0.23; p=0.227), or any other pregnancy, birth, or neonatal outcomes between the Lifestyle Advice or Standard Care groups[201].

2.6 Ultrasound measures – fetal biometry

Ultrasound assessment included measurements of fetal biometry, measured in accordance with national and international standards of practice [209, 210], and consisting of biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC), and femur length (FL).

2.6.1 Head circumference and biparietal diameter

Measurements of HC and BPD were taken from the same image, in a transverse axial section of the fetal head (figure 2.4). The cavum septum pellucidum and thalami were visualised, and the falx cerebri was seen anteriorly and posteriorly. The BPD was measured in centimetres, by placing callipers at the outer edge of the nearer parietal bone to the inner edge of the distal parietal bone[211]. The HC was measured in centimetres, by placing an ellipse around the edge of the outer skull bone [211].

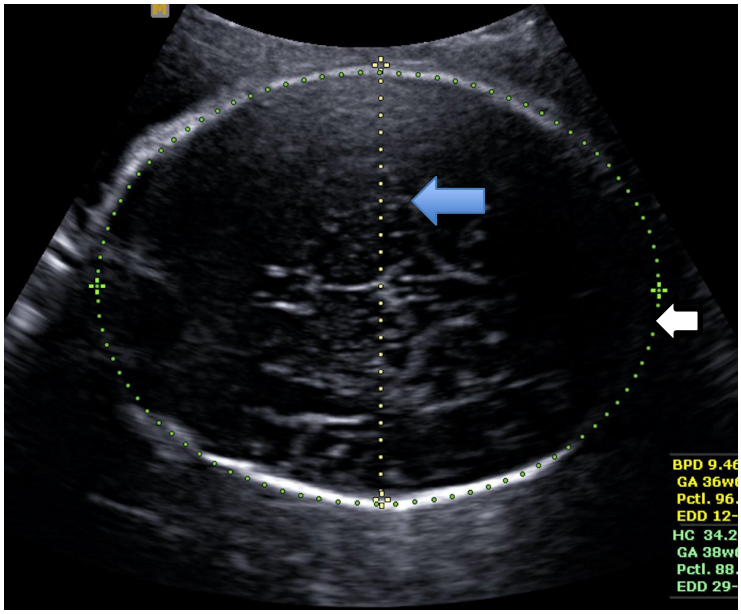


Figure 2.4. Ultrasound image of fetal biparietal diameter (BPD; blue arrow) and head circumference (HC; white arrow). Participant image used with permission.

2.6.2 Abdominal circumference

The AC was measured by obtaining a transverse image of the fetal abdomen at the level of the fetal liver and stomach, including the left portal vein at the umbilicus [211] (figure 2.5). The AC was measured in centimetres.

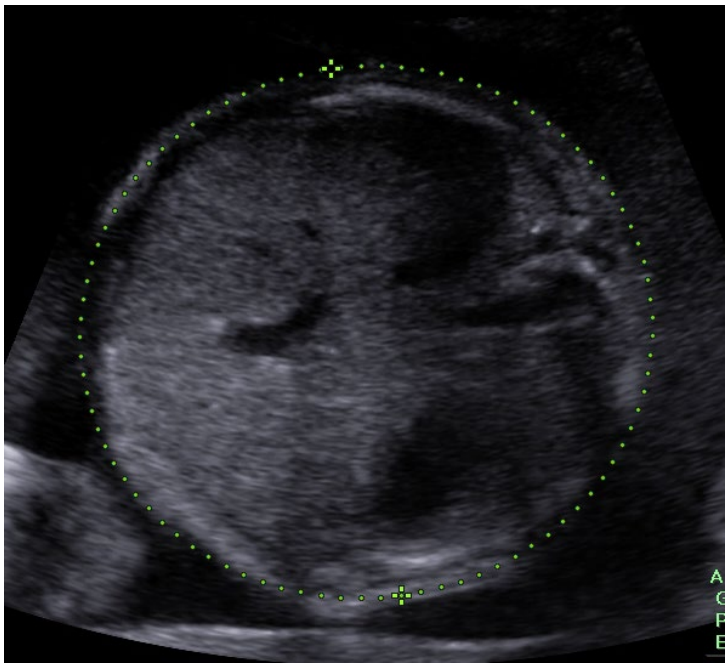


Figure 2.5. Ultrasound image of fetal abdominal circumference (AC). Participant image used with permission.

2.6.3 Femur length

Fetal FL was measured with the fetal thigh in a longitudinal view, and the bone imaged across the ultrasound beam axis and taking up the majority of the view. Callipers were placed along the diaphyseal shaft, excluding the epiphysis, and FL was measured in centimetres (figure 2.6).

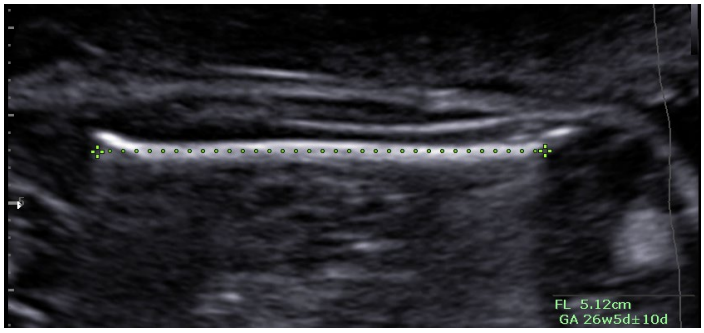


Figure 2.6. Ultrasound image of fetal femur length (FL). Participant image used with permission.

Fetal biometry measures were assessed at three time points: 20, 28, and 36 weeks' gestation. Fetal biometry velocities were calculated as the difference between 28 and 36 week measures, calculated as total change / number of actual days between measurements.

2.6.4 Estimated fetal weight

Estimated fetal weight (EFW) was calculated from standard biometry measures using the Hadlock C formula[211, 212], defined as:

$$EFW\ (gm) = 10^{(1.5662 - 0.0108 \times HC + 0.0468 \times AC + 0.171 \times FL + 0.00034 \times HC \times 10 - 0.003685 \times AC \times FL)}$$

2.6.5 Fetal growth – calculation of fetal biometry z-scores

Fetal biometry measures were converted to z-scores where appropriate reference values existed, to allow for comparison to a standard reference Australian population [211, 213, 214]. Z-scores are calculated by taking the difference between the observed value and the reference mean, then dividing by the reference standard deviation; they therefore represent a measurement in units of reference standard deviations from the reference mean. The reference values are relative to sex and gestational age. Z-scores >0 therefore represent values that are above the reference population mean, while negative z-scores represent values that are below the reference population mean.

Fetal biometry velocity z-scores were also calculated, with reference to fetal growth velocity in a standard reference population [214].

2.7 Ultrasound measures – fetal adiposity

Fetal adiposity measurements obtained included mid-thigh total mass (MTTM), mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM)[118-121, 123, 215].

2.7.1 Mid-thigh total mass, mid-thigh lean mass, and mid-thigh fat mass

Measurements of MTTM, MTLM and MTFM were obtained by taking a longitudinal view of the femur, then rotating the transducer through 90 degrees to obtain a cross-sectional view of the mid-thigh [119, 120]. MTFM was measured by taking the total cross-sectional limb area (MTTM) and subtracting MTLM (consisting of the central lean area comprising muscle and bone) (Figure 2.7).

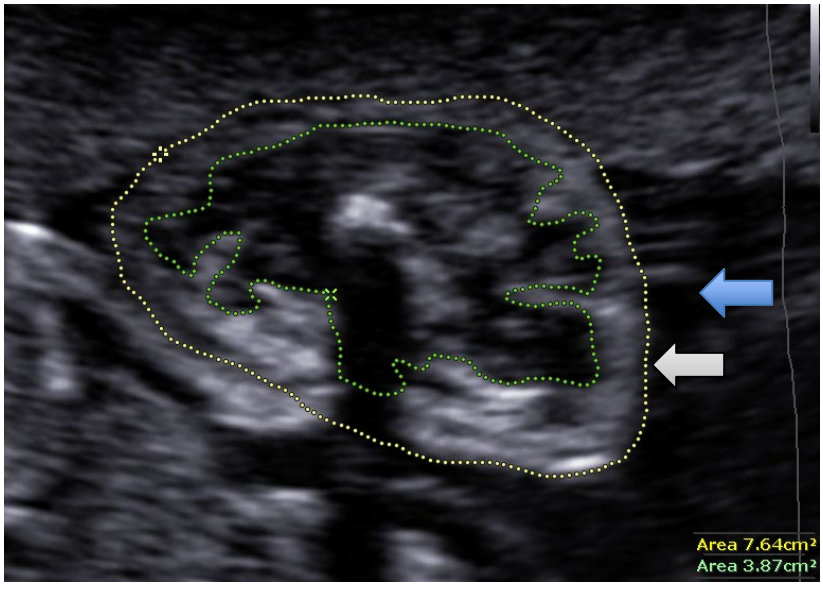


Figure 2.7. Ultrasound image of fetal mid-thigh total mass (MTTM; blue arrow), mid-thigh fat mass (MTFM; white arrow), and mid-thigh lean mass (MTLM). Participant image used with permission.

2.7.2 Abdominal fat mass

Fetal AFM was measured at the same level as for the AC (described above), between fetal mid-axillary lines and anterior to the margins of the ribs (Figure 2.8)[119, 121]. This was measured in millimetres and using magnification.

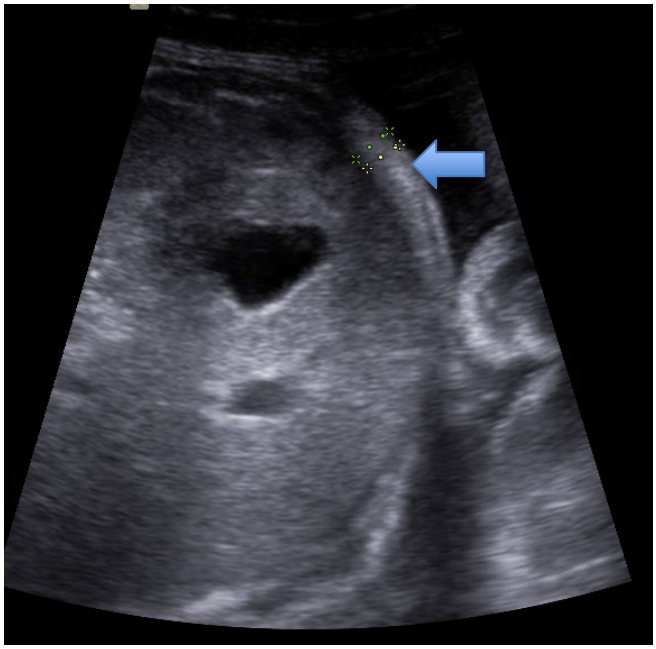


Figure 2.8. Ultrasound image of fetal abdominal fat mass (AFM; blue arrow). Participant image used with permission.

2.7.3 Subscapular fat mass

The SSFM was obtained by a sagittal view of the fetal trunk, to view the entire longitudinal section of the scapula (Figure 2.9). The subcutaneous fat tissue measurement was taken at the level of the end of the scapula [119], and measured in millimetres.

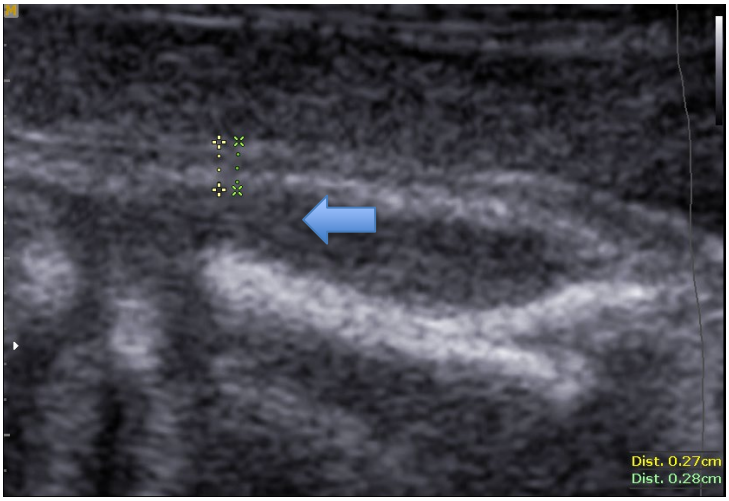


Figure 2.9. Ultrasound image of fetal subscapular fat mass (SSFM; blue arrow). Participant image used with permission.

These measurements have demonstrated good inter-observer variability, including among women who are overweight or obese[118].

Fetal ultrasound measures of adiposity were taken at two time points – 28 and 36 weeks' gestation.

Fetal adiposity velocities were calculated as the difference between 28 and 36 week measures, calculated as total change / number of actual days between measurements.

2.8 Sample size

The sample size available for the analyses were fixed by the number of women who underwent research ultrasounds for at least one time point in the three randomised trials. Additionally, some analyses utilised only results from women randomised to the Standard Care groups of the LIMIT and Optimise randomised trials [188, 201] Each of the three harmonised randomised trials had their own prespecified sample sizes calculated to provide sufficient power for their respective primary outcomes[188, 193, 201]. Detectable difference calculations

relevant to the research questions are presented in each of the chapters that follow.

Chapter 3: Effect of increasing maternal BMI on fetal growth and adiposity

The analyses in this chapter have been accepted and presented as a poster presentation at the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) Virtual World Congress 2021 (Appendix 1).

3.1 Background

While it is well recognised that the infants born to women who are overweight or obese are at an increased risk of being born LGA or with high birthweight, less is known about fetal growth and fetal growth trajectories in this high risk population. In particular, it is unclear whether the growth of fetuses of women who are overweight or obese differs from that of women who are of a normal BMI[182, 185, 216]. Such knowledge has potential implications for timing of antenatal interventions to reduce the risk of infants being born LGA.

What is known?
• Women who are overweight or obese in pregnancy are at an increased risk of delivering an infant with high birthweight, and with increased fat mass
What is not known?
• Which fetal biometry measures differ and when, during pregnancy, fetal growth diverges with increasing maternal BMI • The effect of increasing maternal BMI on fetal subcutaneous fat mass measures over the third trimester
What do these analyses add to previous knowledge?
• There is a continuous, positive linear relationship between increasing maternal BMI and fetal growth measures, with no evidence of threshold effects • The effect of increasing maternal BMI on fetal growth measures is seen as early as 20 weeks' gestation, and impacts fetal growth velocity over the third trimester • Maternal BMI is associated with increased fetal subcutaneous fat measures at 36 weeks', but not 28 weeks' gestation

3.2 Aims

To describe the effect of increasing maternal BMI, across the BMI spectrum, on fetal growth and adiposity.

3.3 Objectives

To describe:

- The effect of maternal BMI on fetal biometry measures and estimated fetal weight;
- The effect of maternal BMI on fetal adiposity measures; and
- The effect of maternal BMI on fetal growth velocity.

3.4 Methods pertaining to this chapter

3.4.1 Description of the cohort

The analyses reported in this chapter use data obtained from research ultrasounds of participants randomised to the Standard Care groups of the LIMIT[199] and Optimise[201] randomised controlled trials.

3.4.2 Detectable difference calculation

The total sample size available for these analyses was 1,377 participants. Calculations were performed for 80% power at one time point, using n=1,121 (the number of 36 week ultrasounds) and n=1,126 (the number of 28 week ultrasounds). The effect of interest was defined as the slope of the regression line, with power to detect a difference from 0 (indicating no relationship). Based on these calculations, the analyses presented have 80% power to detect, at a single time point (either 28 weeks or 36 weeks), a slope of 0.08, corresponding to a change in the fetal measure of 0.08 units for each unit increase in maternal BMI.

3.4.3 Analyses

As the aim of this study was to characterise and estimate the effect of maternal BMI on fetal growth and adiposity measures, a range of statistical analyses were performed. Firstly, to explore whether the relationship between maternal BMI and fetal growth and adiposity measures was linear in nature, and secondly, to estimate this relationship.

3.4.3.1 Analysis 1. What is the nature of the relationship between increasing maternal BMI and fetal growth and adiposity measures?

Fractional polynomial models[217] were used to investigate whether the relationship between maternal BMI and fetal growth and adiposity was best described by a linear, or nonlinear, relationship. Each time point was modelled separately. A series of models were fitted including a predefined set of candidate polynomial transformations allowing for up to two polynomial terms per model, using the *mfp* package[218] in R v.4.0 (R Foundation for Statistical Computing). The models were then compared using the deviance difference compared to a 'full' model, in order to select the 'optimal' model (the model which best balances good fit with parsimony, i.e. fewer parameters). The models were adjusted for maternal age, BMI, parity, smoking and Socioeconomic Indexes for Areas Index of Relative Socioeconomic Disadvantage (SEIFA IRSD) quintile.

3.4.3.2 Analysis 2. What is the effect of increasing maternal BMI on fetal growth and adiposity?

Analyses were performed using linear regression models, with Generalised Estimating Equations (GEEs) to account for repeated measures where relevant. Both adjusted and unadjusted models were fitted, with adjusted models including maternal age, parity, SEIFA IRSD Quintile and smoking status as covariates. For outcomes measured at multiple time points, a time-by-BMI interaction term was included, and the effect of BMI was estimated separately for each time point, regardless of the significance of the interaction term.

Results are presented as the estimated difference in mean fetal growth measure corresponding to a 5 kg/m^2 increase in maternal BMI, and 95% confidence interval, at each time point. The p value for the interaction term is also given (indicated by an asterisk).

3.5 Results

3.5.1 Baseline characteristics

The cohort consisted of 1,377 women who underwent one or more fetal ultrasound assessments. A total of 1,060 women participated in the LIMIT, and 317 women participated in the Optimise randomised trials. Baseline characteristics are presented in Table 3.1, and are similar to the respective full randomised cohorts[188, 201].

There were differences in baseline characteristics between the women in the LIMIT randomised trial and the Optimise randomised trial. Maternal BMI at trial entry differed between the two groups, as a result of the different inclusion criteria of the respective trials (Table 3.1). Women recruited to the Standard Care group of the LIMIT randomised trial were more likely to be in their second or subsequent pregnancy (635 women (59.91%) versus 131 women (41.32%)), report being smokers (122 women (11.51%) versus 13 women (4.10%)), and to be of Caucasian ethnicity (962 women (90.75%) versus 215 women (67.82%)), than women recruited and randomised to the Standard Care group of the Optimise randomised trial (Table 3.1). Approximately half of the women in the Standard Care groups of both randomised trials came from the two most disadvantaged socioeconomic quintiles (569 women (53.68%) versus 151 women (47.64%) (Table 3.1).

Table 3.1 – Participant baseline characteristics: effect of maternal BMI on fetal growth and adiposity

Characteristic	LIMIT Randomised Trial	Optimise Randomised Trial
Overall numbers	1,060	317
BMI (kg/m^2): Mean (SD)	32.40 (5.94)	22.15 (1.66)
BMI category: N(%)		
18.5-24.9	0 (0.00)	317 (100.00)
25.0-29.9	457 (43.11)	0 (0.00)
30.0-34.9	306 (28.87)	0 (0.00)
35.0-39.9	175 (16.51)	0 (0.00)
≥40.0	122 (11.51)	0 (0.00)
Parity: N(%)		
0	425 (40.09)	186 (58.68)
1+	635 (59.91)	131 (41.32)
Smoking Status: N(%)		
Non-smoker	913 (86.13)	302 (95.27)
Smoker	122 (11.51)	13 (4.10)
Missing	25 (2.36)	2 (0.63)
Quintile of Relative Socioeconomic Disadvantage: N(%)		
Q1 (most disadvantaged)	311 (29.34)	58 (18.30)
Q2	258 (24.34)	93 (29.34)
Q3	167 (15.75)	39 (12.30)
Q4	169 (15.94)	76 (23.97)
Q5 (least disadvantaged)	155 (14.62)	51 (16.09)
Ethnicity: N(%)		
Caucasian	962 (90.75)	215 (67.82)
Non-Caucasian	98 (9.25)	102 (32.18)

BMI, body mass index. SD, standard deviation. N, number.

3.5.2 What is the nature of the relationship between increasing maternal BMI and fetal growth and adiposity measures?

Table 3.2 shows the recommended polynomial terms for each outcome at each time point, and each outcome velocity, where applicable. These findings support a linear relationship between maternal BMI and fetal growth and adiposity measures, and fetal growth and adiposity measure z-scores. The model fit was not significantly improved by the addition of nonlinear terms for any outcome at all time points.

Table 3.2 - Recommended polynomial models for fetal growth and adiposity, and growth velocities

Outcome	20 weeks	28 weeks	36 weeks
BPD	2, 3	1	1
HC	3	3, 3	1
FL	1	1	1
AC	1	1	1
EFW	-	1	1
MTLM	-	1	1
MTFM	-	1	1
AFM	-	1	1
SSFM	-	1	-2
BPD z-score	3	1	1
HC z-score	1	0.5, 0.5	3
AC z-score	1	1	1
FL z-score	1	1	1
EFW z-score	-	1	1
BPD velocity	1		
HC velocity	1		
FL velocity	1		
EFW velocity	1		

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. MTLM, mid-thigh lean mass. MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass.

3.5.3 Effect of maternal BMI on fetal growth and biometry measures

Results of linear regression models are presented in Table 3.3, and are presented as estimated difference in means of fetal growth measures corresponding to a 5 kg/m^2 increase in maternal BMI, and 95% confidence interval. There is a positive relationship between maternal BMI and all fetal biometry measures. As EFW is calculated from the aforementioned fetal biometry measures, there is also a positive relationship between maternal BMI and calculated EFW. With regards to the effect of maternal BMI on calculated EFW, for every 5 kg/m^2 increase in maternal BMI, mean EFW increases by 20.76 g (95% CI 7.04, 24.48 g; p=0.003) at 28 weeks' gestation, and by 43.33 g (95% CI 25.20, 61.46 g; p<0.001) at 36 weeks' gestation. In the case of fetal HC, FL, and calculated EFW, this positive relationship with increasing maternal BMI varied with time. The relationship between increasing maternal BMI and fetal AC measurements, however, did not vary significantly with time, and remained statistically significant at all time points assessed.

Table 3.3 – Effect of maternal BMI on fetal growth measures

Outcome	Unadj Est (95% CI)	Unadj p	Adj Est (95% CI)	Adj p
BPD (cm)		0.294*		0.242*
20 Weeks	0.04 (0.02, 0.05)	<0.001	0.04 (0.02, 0.05)	<0.001
28 Weeks	0.02 (0.00, 0.04)	0.020	0.02 (0.00, 0.04)	0.049
36 Weeks	0.03 (0.01, 0.04)	<0.001	0.03 (0.01, 0.04)	0.001
HC (cm)		0.015*		0.013*
20 Weeks	0.14 (0.09, 0.19)	<0.001	0.16 (0.11, 0.21)	<0.001
28 Weeks	0.05 (-0.01, 0.11)	0.103	0.07 (-0.00, 0.13)	0.052
36 Weeks	0.06 (0.01, 0.12)	0.019	0.08 (0.03, 0.14)	0.004
FL (cm)		0.001*		0.001*
20 Weeks	0.04 (0.03, 0.05)	<0.001	0.04 (0.03, 0.05)	<0.001
28 Weeks	0.02 (0.00, 0.03)	0.040	0.01 (-0.00, 0.03)	0.106
36 Weeks	0.01 (-0.01, 0.03)	0.205	0.01 (-0.01, 0.03)	0.271
AC (cm)		0.151*		0.080*
20 Weeks	0.14 (0.09, 0.20)	<0.001	0.14 (0.08, 0.20)	<0.001
28 Weeks	0.12 (0.04, 0.19)	0.004	0.11 (0.02, 0.19)	0.011
36 Weeks	0.20 (0.12, 0.28)	<0.001	0.21 (0.13, 0.29)	<0.001
EFW (g)		0.018*		0.006*
28 Weeks	16.80 (5.24, 28.35)	0.004	20.76 (7.04, 34.48)	0.003
36 Weeks	36.35 (18.99, 53.72)	<0.001	43.33 (25.20, 61.46)	<0.001

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. Unadj Est, unadjusted estimate. Unadj p, unadjusted p value. Adj Est, adjusted estimate. Adj p, adjusted p value. CI, confidence interval. * denotes the p value for the interaction term (a test of whether the relationship differs between time points). Adjusted models included maternal age, parity, SEIFA IRSD Quintile and smoking status as covariates.

3.5.4 Effect of maternal BMI on fetal growth measure z-scores

There is a positive relationship between increasing maternal BMI and all fetal biometry and calculated EFW z-scores, at all time points assessed (Table 3.4). Results are presented as estimated difference in mean fetal biometry measure z-score for an increase in maternal BMI of 5 kg/m^2, and 95% confidence interval. The relationship between increasing maternal BMI and fetal AC z-score showed evidence of change with time, such that, at 20 weeks' gestation, this relationship was not statistically significant (adjusted estimate 0.02 (95% CI -0.02, 0.07), p=0.314), whereas there was a statistically significant positive relationship at both 28 (adjusted estimate 0.08 (95% CI 0.04, 0.12), p<0.001) and 36 weeks' gestation (adjusted estimate 0.15 (95% CI 0.10, 0.19), p<0.001)(Table 3.4). The relationship between increasing maternal BMI and calculated EFW z-score was also statistically significant, and varied with time, having a greater effect size estimate at 36 weeks' (adjusted estimate 0.12 (95% CI 0.08, 0.16), p<0.001) than at 28 weeks' gestation (adjusted estimate 0.08 (95% CI 0.08, 0.16), p<0.001). There was no evidence of the relationship between increasing maternal BMI and fetal BPD z-score, HC z-score, or FL z-score changing with time.

Table 3.4 – Effect of maternal BMI on fetal growth z-scores

Outcome	Unadj Est (95%CI)	Unadj p	Adj Est (95% CI)	Adj p
BPD z-score		0.117*		0.153*
20 Weeks	0.06 (0.02, 0.10)	0.006	0.06 (0.02, 0.11)	0.006
28 Weeks	0.07 (0.01, 0.13)	0.019	0.08 (0.01, 0.14)	0.018
36 Weeks	0.11 (0.06, 0.16)	<0.001	0.11 (0.06, 0.16)	<0.001
HC z-score		0.290*		0.319*
20 Weeks	0.04 (0.01, 0.06)	0.012	0.06 (0.03, 0.09)	<0.001
28 Weeks	0.03 (-0.01, 0.07)	0.177	0.05 (0.01, 0.09)	0.016
36 Weeks	0.06 (0.02, 0.09)	0.003	0.08 (0.04, 0.12)	<0.001
AC z-score		<0.001*		<0.001*
20 Weeks	0.01 (-0.03, 0.06)	0.544	0.02 (-0.02, 0.07)	0.314
28 Weeks	0.07 (0.03, 0.10)	0.001	0.08 (0.04, 0.12)	<0.001
36 Weeks	0.13 (0.09, 0.17)	<0.001	0.15 (0.10, 0.19)	<0.001
FL z-score		0.670*		0.571*
20 Weeks	0.06 (0.04, 0.09)	<0.001	0.07 (0.04, 0.10)	<0.001
28 Weeks	0.05 (0.00, 0.09)	0.031	0.05 (0.01, 0.10)	0.027
36 Weeks	0.05 (0.00, 0.10)	0.036	0.05 (0.01, 0.10)	0.030
EFW z-score		0.041*		0.027*
28 Weeks	0.07 (0.03, 0.11)	<0.001	0.08 (0.04, 0.13)	<0.001
36 Weeks	0.11 (0.07, 0.15)	<0.001	0.12 (0.08, 0.16)	<0.001

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. Unadj Est, unadjusted estimate. Unadj p, unadjusted p value. Adj Est, adjusted estimate. Adj p, adjusted p value. CI, confidence interval. * denotes the p value for the interaction term (a test of whether the relationship differs between time points). Adjusted models included maternal age, parity, SEIFA IRSD Quintile and smoking status as covariates.

3.5.5 Effect of maternal BMI on fetal adiposity measures

The effect of maternal BMI on fetal adiposity measures at 28 and 36 weeks of pregnancy is presented in Table 3.5. Data are presented as estimated difference in means for every 5 kg/m^2 increase in maternal BMI. At 28 weeks' gestation, increasing maternal BMI was associated with a statistically significant positive effect on MTTM and MTLM, but not MTFM, AFM or SSFM (Table 3.5). A significant positive relationship between maternal BMI and measures of fetal adiposity (MTFM, AFM, and SSFM) was observed at 36 weeks' gestation.

Table 3.5 – Effect of maternal BMI on fetal adiposity measures

Outcome	Unadj Est (95% CI)	Unadj p	Adj Est (95% CI)	Adj p
MTTM (cm^2)		0.707*		0.740*
28 Weeks	0.23 (0.11, 0.35)	<0.001	0.22 (0.09, 0.35)	0.001
36 Weeks	0.28 (0.00, 0.56)	0.048	0.27 (-0.01, 0.55)	0.060
MTLM (cm^2)		0.139*		0.130*
28 Weeks	0.17 (0.11, 0.23)	<0.001	0.17 (0.10, 0.24)	<0.001
36 Weeks	0.06 (-0.08, 0.19)	0.389	0.06 (-0.08, 0.19)	0.424
MTFM (cm^2)		0.121*		0.127*
28 Weeks	0.06 (-0.01, 0.14)	0.112	0.05 (-0.03, 0.14)	0.202
36 Weeks	0.22 (0.02, 0.42)	0.035	0.21 (0.00, 0.41)	0.045
AFM (mm)		0.005*		0.006*
28 Weeks	0.04 (-0.02, 0.10)	0.171	0.02 (-0.04, 0.08)	0.580
36 Weeks	0.20 (0.10, 0.30)	<0.001	0.17 (0.07, 0.28)	0.001
SSFM (mm)		<0.001*		<0.001*
28 Weeks	0.01 (-0.04, 0.06)	0.615	-0.01 (-0.07, 0.04)	0.630
36 Weeks	0.28 (0.19, 0.36)	<0.001	0.26 (0.17, 0.35)	<0.001

MTTM, mid-thigh total mass. MTLM, mid-thigh lean mass. MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. Unadj Est, unadjusted estimate. Unadj p, unadjusted p value. Adj Est, adjusted estimate. Adj p, adjusted p value. CI, confidence interval. * denotes the p value for the interaction term (a test of whether the relationship differs between time points). Adjusted models included maternal age, parity, SEIFA IRSD Quintile and smoking status as covariates.

3.5.6 Effect of maternal BMI on fetal growth velocity

The effect of maternal BMI on fetal growth velocity is presented in Table 3.6. Data are presented as mean change in measure for every 5 kg/m^2 increase in maternal BMI. A positive relationship between maternal BMI and BPD, AA, and EFW velocities was observed, but there was no evidence of a relationship with FL or HC velocity (Table 3.6).

Table 3.6 – Effect of maternal BMI on fetal biometry and estimated fetal weight velocities

Outcome	Unadj Est (95% CI)	Unadj p	Adj Est (95% CI)	Adj p
BPD Velocity (cm/wk)	0.003 (0.001, 0.004)	0.005	0.002 (0.000, 0.004)	0.026
HC Velocity (cm/wk)	0.005 (-0.002, 0.011)	0.139	0.005 (-0.003, 0.012)	0.205
FL Velocity (cm/wk)	0.000 (-0.001, 0.002)	0.605	-0.000 (-0.002, 0.001)	0.766
AA Velocity (cm^2/wk)	0.111 (0.072, 0.150)	<0.001	0.110 (0.066, 0.155)	<0.001
EFW Velocity (g/wk)	4.360 (2.837, 5.884)	<0.001	4.409 (2.704, 6.115)	<0.001

BPD, biparietal diameter. HC, head circumference. FL, femur length. AA, abdominal area. EFW, estimated fetal weight. Unadj Est, unadjusted estimate. Unadj p, unadjusted p value. Adj Est, adjusted estimate. Adj p, adjusted p value. CI, confidence interval.

3.5.7 Effect of maternal BMI on fetal growth velocity z-scores

The effect of increasing maternal BMI on fetal growth velocity z-scores is presented in Table 3.7. Data are presented as change in the measure for every 5 kg/m^2 increase in maternal BMI. As with fetal growth velocity, increasing maternal BMI was associated with increasing BPD, AA, and EFW velocity z-scores. There was no evidence of a relationship between increasing maternal BMI and FL or HC velocity z-score.

Table 3.7 – Effect of maternal BMI on fetal biometry and estimated fetal weight velocity z-scores

Outcome	Unadj Est (95% CI)	Unadj p	Adj Est (95% CI)	Adj p
BPD Velocity z-score	0.066 (0.021, 0.111)	0.004	0.072 (0.021, 0.123)	0.006
HC Velocity z-score	0.005 (-0.002, 0.011)	0.139	0.005 (-0.003, 0.012)	0.205
FL Velocity z-score	0.013 (-0.035, 0.061)	0.598	0.000 (-0.053, 0.054)	0.987
AA Velocity z-score	0.106 (0.068, 0.145)	<0.001	0.109 (0.065, 0.153)	<0.001
EFW Velocity z-score	0.132 (0.086, 0.178)	<0.001	0.134 (0.082, 0.185)	<0.001

BPD, biparietal diameter. HC, head circumference. FL, femur length. AA, abdominal area. EFW, estimated fetal weight. Unadj Est, unadjusted estimate. Unadj p, unadjusted p value. Adj Est, adjusted estimate. Adj p, adjusted p value. CI, confidence interval.

3.6 Discussion

These analyses identify a positive linear relationship between increasing maternal BMI and measures of fetal growth, and measures of fetal adiposity beyond 28 weeks' gestation. There is also a strong positive relationship with maternal BMI for most fetal biometry measure velocities over the course of pregnancy.

These findings are consistent with published literature to date. Specifically, the findings demonstrate increasing maternal BMI is associated with increasing fetal biometry measures evident from 20 weeks' gestation. Sovio and colleagues[182] have reported similar findings, where the rate of abdominal circumference measurements greater than the 90th percentile was higher among women who were obese, compared with women who were not obese, as early as 20 weeks' gestation. While this group only reported on abdominal circumference (AC) and head circumference (HC), the current analyses extend this to include additional fetal biometry measures (FL and BPD), and estimated fetal weight (EFW). Ay and colleagues[184] have also demonstrated in Dutch women in the highest BMI quintile, estimated fetal weight z-scores were higher from mid-pregnancy, compared with women with a BMI in the reference BMI quintile (3rd quintile). Similarly, Zhang and colleagues identified increased fetal biometry measures from 21 weeks' gestation among women who were obese, in comparison to fetuses of women who were of a normal BMI or overweight[185].

In the current analyses maternal BMI was considered as a continuous variable, demonstrating a linear relationship between maternal BMI and fetal growth measures, with no threshold effects. Previously published studies have considered maternal BMI as a categorical variable[182, 184, 185, 219], with categorisation reducing statistical power. These studies also had relatively small numbers of women at the extremes of maternal BMI[182, 184, 185], and therefore may have been underpowered to characterise the relationship between maternal BMI and fetal growth measures.

Consideration of when, over the course of pregnancy, maternal BMI begins to exert an effect on fetal growth measures is of importance in considering antenatal interventions, as most interventions commence in the late first or early second trimester[188, 220, 221]. The ultrasound findings reported here suggest divergence of fetal growth from as early as 20 weeks' gestation, thus interventions commencing in very early pregnancy, or targeting the preconception period, may be more effective.

Furthermore, infant anthropometry has been adopted as a surrogate marker of intrauterine fat deposition and has not considered longitudinal patterns of adipose accumulation[112, 222, 223]. The findings presented here are the first to investigate the effect of maternal BMI on measures of fetal adiposity over the course of pregnancy, demonstrating increasing maternal BMI is significantly associated with increasing fetal measures of abdominal fat mass, subscapular fat mass, and mid-thigh fat mass, at 36 weeks' gestation, but not 28 weeks' gestation. The third trimester of pregnancy is a time of rapid and proportionally increasing fetal fat deposition[224]. Thus it is possible that 28 weeks gestation is too early to see clinically relevant differences in fetal subcutaneous tissue fat thickness measures.

Previous work investigating fetal fat deposition by ultrasound subcutaneous tissue fat thickness measures has comprised small numbers of women, who have largely been diagnosed with pregestational[225] or gestational diabetes[119, 226, 227]. Per the Pederson hypothesis, maternal hyperglycaemia increases placental glucose transport that, via fetal production of insulin, stimulates increased fetal growth and fat deposition[134]. While this relationship has been shown among women with pregestational or gestational diabetes, it is plausible that alternative pathways to hyperglycaemia may be acting to promote fetal growth and fat deposition in the setting of maternal overweight and obesity.

Chapter 4: The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight or obese

The analyses reported in this chapter have been presented as a poster presentation at the Royal College of Obstetrics and Gynaecology (RCOG) World Congress, 2017, and published in the British Journal of Obstetrics and Gynaecology[228]. The poster and published manuscript are included as appendices 2 and 3.

4.1 Background

Women who are overweight or obese in pregnancy are at an increased risk of adverse pregnancy outcomes that may act in combination with GDM to affect fetal growth and adiposity. GDM is defined as carbohydrate intolerance diagnosed during pregnancy, increases in prevalence with increasing maternal BMI[56], and is independently associated with adverse perinatal outcomes[143, 154, 155], including an increased risk of birth of an infant LGA or of high birth weight. Maternal overweight or obesity and GDM commonly coexist, and have many similar metabolic and endocrine characteristics[168, 229].

It is unclear whether the combination of maternal overweight or obesity and GDM represents an additive risk in pregnancy, and whether diagnosis and treatment of GDM among women who are overweight or obese modifies this risk. It is also unclear how much of the increased risk of adverse outcomes attributable to maternal overweight or obesity reflects the increased prevalence of GDM. While both conditions have independent effects on fetal growth, maternal overweight or obesity may confer additional risk of adverse outcome.

What is known?
• Both maternal overweight and obesity, and GDM, are independently associated with an increased risk of delivering an infant with high birthweight, and with increased neonatal fat mass
What is not known?
• The relative contribution, and combined effects, of GDM and increasing maternal BMI on fetal growth and adiposity
What do these analyses add to previous knowledge?
• Increasing maternal BMI is directly associated with increasing fetal biometry measures • There is no evidence of an additional mediated effect due to diagnosed and treated GDM • Among women who are overweight or obese, there is no evidence that either higher BMI category or diagnosed and treated GDM is associated with ultrasound measures of fetal adiposity

4.2 Aim

To evaluate, among pregnant women who are overweight or obese, the degree to which the association between maternal BMI and fetal growth and adiposity is mediated via diagnosed and treated GDM

4.3 Objectives

The objectives of this chapter are to describe, among women who are overweight or obese in early pregnancy:

- The mediating effect of diagnosed and treated GDM on fetal biometry
- The mediating effect of diagnosed and treated GDM on fetal adiposity measures
- The mediating effect of diagnosed and treated GDM on fetal biometry measure velocity; and
- The mediating effect of diagnosed and treated GDM on fetal adiposity measure velocity

4.4 Methods pertaining to this chapter

4.4.1 Description of the cohort

The analyses reported in this chapter use data obtained from research ultrasounds of participants from the Standard Care group of the LIMIT randomised trial[188], who also had data on GDM diagnosis.

4.4.2 Detectable difference calculation

The sample size for these analyses was 900 participants in the Standard Care group of the LIMIT trial. Of the 1,104 women randomised to the Standard Care group, 900 women underwent one or more ultrasound scans and had GDM status recorded (Figure 2.1). As mediation analyses such as those presented here are most commonly secondary exploratory analyses, detectable difference calculations are not usually performed. Nevertheless, to ascertain how much power there was to detect biologically plausible mediation effects, simulation studies were run. These simulation studies were used to determine the power to detect the presence of indirect (i.e. mediated via GDM) effects ranging from 10% to 90% of the total effect on the outcome of interest. The Stata program paramed was used to estimate this effect[230, 231]. Overall, the simulation studies identified that there was very low power to detect anything but very large indirect effects (and therefore presence of mediation). It is likely that only analyses involving many thousands of participants would be adequately powered to detect anything other than very large indirect effects. However, the effect estimates presented here are considered valid estimates and patterns across the different outcomes may suggest whether or not mediation is present.

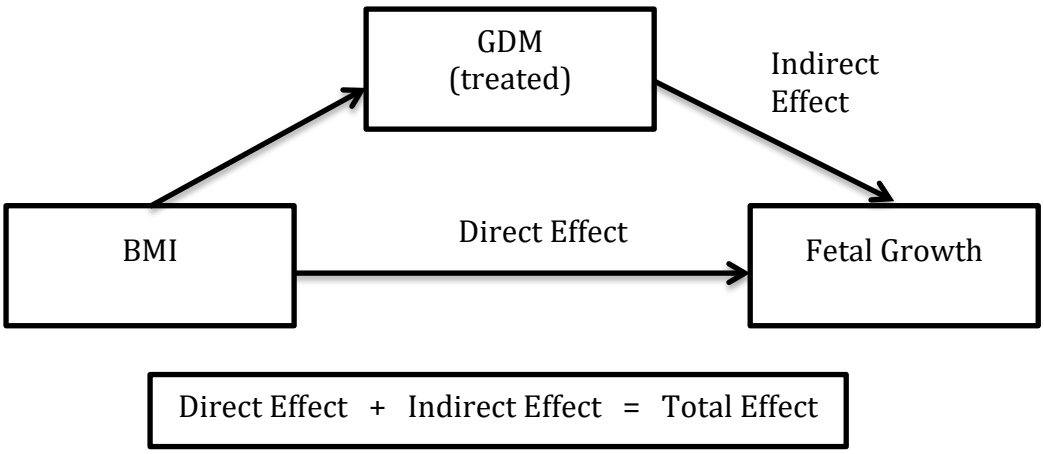
4.4.3 Statistical analysis

Regression-based mediation analyses were performed to investigate the extent to which any effect of maternal BMI on fetal biometry and adiposity measures at 36 weeks' gestation were mediated via diagnosed and treated GDM. For the purposes of analysis, BMI was divided into 2 categories (25.0-29.9 kg/m^2 and

≥30.0 kg/m^2). Regression-based causal mediation models using an extension of the Baron-Kenny method[230] were fitted to determine the effect of BMI category, and any mediating effect of diagnosed and treated GDM, on measures of fetal growth and adiposity, resulting in 3 estimates for each outcome (see Figure 4.1):

- Total Effect i.e. the total effect of BMI ≥30.0 (versus BMI 25.0-29.9 kg/m^2) on the outcome, including any effect that occurs due to the increased risk of a diagnosis of GDM;
- Direct Effect i.e. the effect of BMI ≥30.0 kg/m^2, versus BMI 25.0-29.9 kg/m^2, on the outcome, excluding any effect that occurs via the effect of BMI on risk of diagnosis of GDM; and
- Indirect Effect i.e. the effect of BMI ≥30.0 kg/m^2 on the outcome which occurs via its effect on GDM (i.e. via increased risk of GDM).

Figure 4.1. Hypothesised relationship between maternal BMI, GDM diagnosis, and fetal growth and adiposity



In summary, effect estimates represent the difference in mean outcome values at 36 weeks' gestation between BMI categories. Diagnosed and treated GDM was investigated as a potential mediator, that is, a factor that lies along the causal pathway from increased maternal BMI to effect on fetal growth. Maternal BMI was the direct effect, diagnosed and treated GDM was the mediator, and the total effect was the combination of the effect of BMI along all causal pathways other than GDM, and the effect of maternal BMI via the effect of GDM.

Analyses were adjusted for hospital, parity, SEIFA IRSD Quintile, maternal smoking and maternal age at consent. All analyses were performed using the *paramed* program in Stata v14 (StataCorp, College Station, Texas, USA)[230, 231].

4.5 Results

4.5.1 Baseline characteristics

These results pertain to the 1,104 women randomised to the Standard Care group of the LIMIT study, of whom 912 had at least one research ultrasound performed at 28 and/or 36 weeks of gestation. Twelve women were excluded from mediation analyses as GDM status was missing (11 women) or ultrasound data were missing for all measures used in these analyses (one woman; Figure 2.1). Baseline characteristics of participants are presented in Table 4.1.The mean BMI of women at trial entry was 32.56 (SD 5.95) kg/m^2, with a diagnosis of GDM made in 102 women (11.33%). The majority of women were of Caucasian ethnicity [825 women (91.67%)], in their second or subsequent pregnancy [534 women (59.33%)], and were non-smokers [782 women (86.89%)]. These are similar to the baseline characteristics of the full recruited and randomised cohort[188].

Table 4.1 – Participant baseline characteristics: the mediating effect of GDM on fetal growth and adiposity in women who are overweight or obese

Characteristic	LIMIT Standard Care
Overall numbers	900
BMI (kg/m²): Mean (SD)	32.56 (5.95)
BMI Category (kg/m²): N(%)	
25.0-29.9	372 (41.33)
30.0-34.9	269 (29.89)
35.0-39.9	152 (16.89)
≥40.0	107 (11.89)
Diagnosis of GDM: N(%)	
No	798 (88.67)
Yes	102 (11.33)
Parity: N(%)	
0	366 (40.67)
1+	534 (59.33)
Smoking Status: N (%)	
Non-smoker	782 (86.89)
Smoker	99 (11.00)
Missing	19 (2.11)
Quintile of Relative Socioeconomic Disadvantage: N (%)	
Q1 (most disadvantaged)	260 (28.89)
Q2	220 (24.44)
Q3	142 (15.78)
Q4	142 (15.78)
Q5 (least disadvantaged)	136 (15.11)
Ethnicity: N (%)	
Caucasian	825 (91.67)
Non-Caucasian	75 (8.33)

BMI, body mass index. SD, standard deviation. N, number. GDM, gestational diabetes, Q, quintile.

4.5.2 Effect of maternal BMI, and diagnosed and treated GDM, on fetal biometry measures at 36 weeks' gestation

Results of this analysis are presented in Table 4.2. Among women who were overweight or obese in early pregnancy, higher maternal BMI category was directly associated with higher HC, AC, and EFW z-scores (direct effect). However, there was no evidence of an additional mediated effect due to diagnosed and treated GDM on any of these fetal biometry, or EFW, z-scores (indirect effect).

Table 4.2 – Effects of maternal BMI and GDM, on fetal biometry

Outcome	Unadj (95% CI)	Unadj p	Adj (95% CI)	Adj p
BPD z-score				
Total Effect	0.13 (-0.05, 0.30)	0.156	0.10 (-0.08, 0.28)	0.271
Direct Effect	0.14 (-0.03, 0.30)	0.104	0.11 (-0.06, 0.28)	0.198
Indirect Effect	-0.01 (-0.03, 0.01)	0.266	-0.01 (-0.03, 0.01)	0.315
HC z-score				
Total Effect	0.16 (0.03, 0.29)	0.017	0.17 (0.02, 0.31)	0.018
Direct Effect	0.17 (0.05, 0.29)	0.007	0.18 (0.05, 0.31)	0.005
Indirect Effect	-0.01 (-0.02, 0.01)	0.223	-0.01 (-0.02, 0.00)	0.183
AC z-score				
Total Effect	0.25 (0.10, 0.40)	0.001	0.26 (0.11, 0.42)	0.001
Direct Effect	0.25 (0.10, 0.40)	0.001	0.26 (0.11, 0.41)	0.001
Indirect Effect	0.01 (-0.01, 0.02)	0.466	0.01 (-0.01, 0.02)	0.532
FL z-score				
Total Effect	0.11 (-0.04, 0.26)	0.150	0.09 (-0.06, 0.24)	0.244
Direct Effect	0.11 (-0.04, 0.25)	0.159	0.09 (-0.06, 0.24)	0.256
Indirect Effect	0.00 (-0.01, 0.02)	0.793	0.00 (-0.01, 0.02)	0.832
EFW z-score				
Total Effect	0.22 (0.08, 0.35)	0.002	0.22 (0.08, 0.35)	0.002
Direct Effect	0.21 (0.08, 0.35)	0.002	0.22 (0.08, 0.35)	0.002
Indirect Effect	0.00 (-0.01, 0.02)	0.790	0.00 (-0.01, 0.02)	0.901

BPD, biparietal diameter. HC, head circumference. AC, abdominal circumference. FL, femur length. EFW, estimated fetal weight. Unadj, unadjusted. Adj, adjusted. CI, confidence interval. P, p value. Analyses were adjusted for centre, parity, SEIFA IRSD quintile, maternal smoking status, and maternal age at consent.

4.5.3 Effect of maternal BMI, and diagnosed and treated GDM, on fetal adiposity measures at 36 weeks' gestation

Results of this analysis are presented in Table 4.3. Among women who were overweight or obese in early pregnancy, higher maternal BMI category was neither directly nor indirectly (via diagnosed and treated GDM), associated with a significant effect on any measures of subcutaneous fetal adiposity. All possible effect estimates were small, and 95% confidence intervals crossed zero, thus a clinically relevant effect is unlikely.

Table 4.3 – Effects of maternal BMI and GDM, on fetal adiposity

Outcome	Unadj (95% CI)	Unadj p	Adj (95% CI)	Adj p
MTFM (cm^2)				
Total Effect	0.17 (-0.37, 0.71)	0.542	0.07 (-0.50, 0.64)	0.802
Direct Effect	0.14 (-0.38, 0.65)	0.604	0.04 (-0.49, 0.58)	0.877
Indirect Effect	0.03 (-0.03, 0.10)	0.308	0.03 (-0.03, 0.10)	0.352
AFM (mm)				
Total Effect	0.22 (-0.09, 0.53)	0.157	0.18 (-0.11, 0.48)	0.223
Direct Effect	0.20 (-0.08, 0.47)	0.159	0.17 (-0.11, 0.45)	0.236
Indirect Effect	0.03 (-0.01, 0.06)	0.157	0.01 (-0.02, 0.05)	0.402
SSFM (mm)				
Total Effect	0.32 (-0.12, 0.38)	0.316	0.12 (-0.14, 0.39)	0.362
Direct Effect	0.13 (-0.11, 0.38)	0.291	0.13 (-0.12, 0.39)	0.306
Indirect Effect	-0.01 (-0.04, 0.02)	0.689	-0.01 (-0.04, 0.02)	0.508

MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. Unadj, unadjusted. Adj, adjusted. CI, confidence interval. P, p value. Analyses were adjusted for centre, parity, SEIFA IRSD quintile, maternal smoking status, and maternal age at consent.

4.5.4 Effect of maternal BMI, and diagnosed and treated GDM, on fetal growth velocity

Results of these analyses are presented in Table 4.4. Among women who were overweight or obese in early pregnancy, there was no significant direct effect of higher maternal BMI category and fetal growth velocity for any of the measures assessed, or EFW. There was no evidence of a mediated effect due to diagnosed and treated GDM on any of the fetal biometry measure velocity or EFW velocity z-scores in the cohort.

Table 4.4 – Effect of maternal BMI and GDM, on fetal biometry velocity

Outcome	Unadj (95% CI)	Unadj p	Adj (95% CI)	Adj p
BPD Velocity z-score				
Total Effect	0.08 (-0.07, 0.23)	0.301	0.07 (-0.08, 0.23)	0.364
Direct Effect	0.08 (-0.06, 0.23)	0.262	0.08 (-0.07, 0.23)	0.296
Indirect Effect	-0.01 (-0.02, 0.01)	0.520	-0.01 (-0.03, 0.01)	0.396
HC Velocity z-score				
Total Effect	0.003 (-0.00, 0.01)	0.107	0.002 (-0.00, 0.01)	0.176
Direct Effect	0.003 (-0.00, 0.01)	0.088	0.002 (-0.00, 0.01)	0.145
Indirect Effect	-0.000 (-0.00, 0.00)	0.518	-0.000 (-0.00, 0.00)	0.511
AA Velocity z-score				
Total Effect	0.08 (-0.05, 0.21)	0.239	0.10 (-0.04, 0.23)	0.160
Direct Effect	0.08 (-0.05, 0.21)	0.246	0.10 (-0.04, 0.23)	0.165
Indirect Effect	0.00 (-0.01, 0.02)	0.923	0.00 (-0.02, 0.02)	0.961
FL Velocity z-score				
Total Effect	-0.01 (-0.17, 0.15)	0.892	-0.02 (-0.18, 0.14)	0.829
Direct Effect	-0.01 (-0.18, 0.15)	0.878	-0.02 (-0.18, 0.14)	0.818
Indirect Effect	0.00 (-0.02, 0.02)	0.878	0.00 (-0.02, 0.02)	0.896
EFW Velocity z-score				
Total Effect	0.14 (-0.02, 0.29)	0.086	0.13 (-0.02, 0.29)	0.095
Direct Effect	0.14 (-0.02, 0.29)	0.084	0.14 (-0.02, 0.30)	0.089
Indirect Effect	-0.00 (-0.02, 0.02)	0.890	-0.00 (-0.02, 0.02)	0.780

BPD, biparietal diameter. HC, head circumference. AC, abdominal circumference. FL, femur length. EFW, estimated fetal weight. Unadj, unadjusted. Adj, adjusted. CI, confidence interval. P, p value. Analyses were adjusted for centre, parity, SEIFA IRSD quintile, maternal smoking status, and maternal age at consent.

4.5.5 Effect of maternal BMI, and diagnosed and treated GDM, on fetal adiposity velocity

Results of these analyses are presented in Table 4.5. Among women who were overweight or obese in early pregnancy, there was no significant direct effect of maternal BMI category and fetal adiposity measure velocity in any of the subcutaneous tissue fat thickness measurements performed, and no mediation effect by diagnosed and treated GDM. Again, all effect estimates were small, and 95% confidence intervals crossed zero, making a true, clinically relevant, effect unlikely.

Table 4.5 – Effect of maternal BMI and GDM, on fetal adiposity velocity

Outcome	Unadj (95% CI)	Unadj p	Adj (95% CI)	Adj p
MTFM Velocity				
Total Effect	0.004 (-0.007, 0.015)	0.474	0.003 (-0.008, 0.015)	0.603
Direct Effect	0.003 (-0.007, 0.013)	0.547	0.002 (-0.008, 0.012)	0.685
Indirect Effect	0.001 (-0.000, 0.002)	0.138	0.001 (-0.000, 0.002)	0.173
AFM Velocity				
Total Effect	0.003 (-0.004, 0.009)	0.427	0.003 (-0.003, 0.009)	0.385
Direct Effect	0.002 (-0.003, 0.007)	0.490	0.002 (-0.003, 0.008)	0.424
Indirect Effect	0.001 (-0.000, 0.001)	0.096	0.000 (-0.000, 0.001)	0.212
SSFM Velocity				
Total Effect	0.004 (-0.001, 0.010)	0.136	0.005 (-0.002, 0.011)	0.150
Direct Effect	0.005 (-0.001, 0.010)	0.079	0.005 (-0.000, 0.011)	0.065
Indirect Effect	-0.000 (-0.001, 0.000)	0.243	-0.001 (-0.001, 0.000)	0.170

MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. Unadj, unadjusted. Adj, adjusted. CI, confidence interval. P, p value. Analyses were adjusted for centre, parity, SEIFA IRSD quintile, maternal smoking status, and maternal age at consent.

4.6 Discussion

In a population of overweight and obese women, while higher maternal BMI category was directly associated with an increase in fetal biometry measures, there was no evidence of an additional mediated effect due to diagnosed and treated GDM. There was no evidence that maternal BMI category was directly or indirectly (via diagnosed and treated GDM) associated with ultrasound measures of fetal adiposity.

While maternal overweight or obesity and GDM are both recognised risk factors for fetal overgrowth and birth weight >4kg[56, 154, 232], their relative contribution and combined effects on fetal growth has been uncertain. In a secondary analysis of the HAPO study[168], both maternal obesity and GDM were independently associated with infant birth weight and adiposity. Notably, the combination of both maternal obesity and GDM resulted in a greater risk than either factor alone, suggesting the effect of maternal BMI is not entirely due to the mediating effect of GDM[168]. This was an observational study, in which clinicians were blinded to maternal glucose tolerance test results, with treatment only offered when blood glucose concentrations were extremely elevated. In contrast, the data presented in this chapter include all women offered screening, and those with an abnormal glucose tolerance test, diagnostic of GDM, subsequently receiving treatment according to state-wide guidelines.

Schaefer-Graf and colleagues investigated the correlation between maternal BMI, maternal glucose concentrations and fetal and newborn large for gestational age status, in a group of women of all BMI ranges, who were offered treatment following a diagnosis of GDM or impaired glucose tolerance[186]. While the risk of a LGA infant was significantly higher among women who were obese, this did not appear to be attributable to differences in maternal blood glucose concentrations[186]. Although this was a relatively small retrospective cohort of women, the findings are consistent with the current analyses, providing further evidence to support the clinical importance of treatment of GDM in this high-risk population.

The Pedersen hypothesis of fetal overgrowth[135] suggests that infant macrosomia among women with diabetes reflects the effects of fetal hyperglycaemia and subsequent hyperinsulinaemia, resulting from maternal hyperglycaemia. The net effect of increasing birth weight is attributable to the growth-promoting effects of both insulin and glucose. Furthermore, there is strong evidence to indicate that appropriate treatment and adequate control of blood glucose concentrations in women diagnosed with GDM ameliorates these effects on fetal growth[141, 142].

It is possible that the current findings represent "saturation" in the direct effect of maternal BMI on fetal biometry and adiposity, and that additional effects of treated GDM in an already disordered intra-uterine milieu as observed in women who are overweight or obese, results in no discernable additional effects. Fetuses of women who are overweight or obese are more likely to have abdominal circumferences >90th percentile as early as 20 weeks' gestation, predating the diagnosis and potential effects of GDM[182]. It has been reported that fetuses of women who are overweight or obese are consistently larger than average, for all ultrasound biometry measures, when using standard population based growth charts[118]. Data presented here further support that maternal overweight and obesity is associated with an early, substantial effect on fetal growth, which is not further exacerbated by diagnosed and treated GDM.

There is growing evidence that maternal overweight and obesity influences fetal growth through pathways beyond glucose transport. Obesity is a complex condition in which multiple metabolic pathways are altered and adipose tissue represents a metabolically active tissue[233]. Metabolic factors including circulating triglycerides[180, 234], leptin[223, 235, 236] and adiponectin[68, 237] have been associated with higher infant birth weight and adiposity, specifically in women who are overweight or obese, and in women with GDM. The associations with, and relative contributions of these factors to fetal fat accumulation, however, are not yet known. These results may represent an effect of maternal

overweight or obesity on fetal growth operating outside of the glucose/insulin pathway.

The findings presented reveal no evidence that maternal BMI of 30 kg/m^2 or more (compared to BMI 25.0-29.9 kg/m^2) has a direct or indirect (via diagnosed and treated GDM) effect on ultrasound measures of fetal adiposity measures at 36 weeks' gestation. While not directly comparable with the population of women reported here, and using different fetal ultrasound measures of adiposity, Akiba and colleagues showed that the fetuses of women with GDM had statistically significantly greater fractional arm volumes, compared with fetuses of women with normal glucose tolerance, from 32 weeks' gestation[238]. The cohort of women reported on by Akiba and colleagues were predominantly of a normal BMI[238] and had fewer numbers, increasing the risk of type 1 and type 2 error in their findings, with very large numbers required for such mediation analyses.

These analyses, and those presented in chapter 3, provide important information on the effects of maternal BMI on fetal biometry, with increased maternal BMI associated with increased fetal growth. However, an association between increased maternal BMI category and fetal adiposity measures, in this cohort of women who were overweight or obese in early pregnancy was not identified. While this may seem in contrast to the findings on association between maternal BMI and fetal growth and biometry (chapter 3 of this thesis), it is likely a result of the current analyses being confined to women who were overweight or obese in early pregnancy, a smaller sample size, and use of BMI categories rather than BMI as a continuous variable. Furthermore, the effect of increasing maternal BMI category on fetal biometry and adiposity was not shown to be due to its effect on diagnosed and treated GDM. While this may reflect "saturation" in the risk attributable to the direct effects of BMI on fetal growth, it may also represent the effects of a universal policy of screening for and treatment of GDM.

Chapter 5: Exploring fetal growth of LGA and non-LGA infants

The analyses reported in this chapter have been submitted for peer review and publication to JAMA Network Open. The submitted manuscript is included as appendix 4.

5.1 Background

The analyses in this chapter will consider fetal growth and adiposity, and fetal growth patterns, of infants born large for gestational age (LGA). An understanding of the fetal growth patterns of infants born LGA may allow for targeting of interventions during pregnancy, in particular timing, to prevent aberrant growth. While fetal growth has been studied in infants born LGA to women with diabetes[239-241], there is little published work on fetal growth of infants born LGA to women who do not have diabetes.

What is known?
Infants born LGA are at an increased risk of both short and long term adverse outcomes.
What is not known?
During pregnancy, when fetal growth and fetal growth patterns diverge among infants born LGA, compared with those who are not born LGA.
What do these analyses add to previous knowledge?
Infants born LGA have larger fetal biometry measures, and higher fetal growth trajectories, from as early as 20 weeks' gestation. Infants born LGA have consistently larger fetal adiposity measures from 28 weeks' gestation, and these differences increase over time.

5.2 Aim

To determine whether the discrepancy in fetal growth and adiposity measures, between infants born LGA and those born not LGA is primarily due to a larger size throughout gestation, or reflect different trajectories of growth.

5.3 Objectives

The objectives of this chapter are to compare across pregnancy between infants born LGA and those who are not:

- differences in fetal growth measures
- differences in fetal adiposity measures; and
- the impact of maternal BMI on fetal growth patterns.

5.4 Methods pertaining to this chapter

5.4.1 Description of the cohort

The analyses reported in this chapter use data obtained from research ultrasounds of participants in three harmonised randomised controlled trials, the LIMIT[199], GRoW[193] and Optimise[201] trials, from both Standard Care and intervention groups. The relationship between fetal growth and adiposity and LGA was not expected to differ according to intervention (or any other baseline characteristics), thus it was considered appropriate to use participants from both groups in order to maximise statistical power.

5.4.2 Fetal ultrasound measures

Fetal biometry measures were obtained as described previously (Chapter 2), and included measurements of fetal BPD, HC, AC, and FL. From these measurements, EFW was calculated, as previously described (Chapter 2). Fetal biometry measures, and calculated EFW, were converted to z-scores, using a standard reference Australian population chart[211, 213, 214], as previously described (Chapter 2).

5.4.3 Definition of LGA

Infants were considered large-for-gestational age (LGA) if birth weight was >90th percentile for gestational age and infant sex[242].

5.4.4 Detectable effect calculation

The total available sample size for these analyses was all participants from all three studies who had a fetal ultrasound measure for at least one time point. A total of 3,260 women were included. The overall rate of LGA in this cohort was 17.58%. As a conservative estimate, 2,700 participants at a single time point, with the assumed rate of LGA, gives 80% power (with two-sided alpha 0.05) to detect a difference of approximately 0.12 SD in fetal biometry and adiposity measures between LGA and non-LGA infants.

For combined LGA and time effects (i.e. a difference between LGA and non-LGA infants in fetal measures at individual time points, and also a change in this difference over time), data were simulated using a range of effect sizes. The sample size was set at 2,700, with two time points, and an LGA rate of 17.58%. Based on 1,000 simulations (utilising a linear regression model with time-by-LGA interaction term and Generalised Estimating Equations to account for correlation due to repeated measures), the power to detect the range of effect sizes was >80% to detect >0.1 SD of difference between LGA and non-LGA, and time-by-LGA interaction effects between 0.5x and 0.75x those of the LGA effect.

5.4.5 Analysis 1: 2-way interaction models

These analyses investigated whether differences in fetal biometry measures between LGA infants and non-LGA infants were evident from 20 weeks' gestation, or whether diverging growth trajectories occur later in pregnancy. Fetal biometry measures at 20, 28, and 36 weeks' gestation; fetal adiposity measures at 28 and 36 weeks' gestation; and fetal growth velocities were available for analysis. Linear regression models were constructed, with LGA, time and their interaction, with GEEs to account for correlation due to repeated measures. Models were additionally adjusted for maternal BMI, parity, age, intervention group, smoking status, and quintile of socioeconomic disadvantage. A test of interaction between LGA status and time was performed for each growth measure, and the mean difference between LGA and non-LGA infants was estimated separately for each time point. Results are presented as difference in means (LGA – non-LGA), and 95% Confidence Interval.

5.4.6 Analysis 2: 3-way interaction models

Based on the results of the above 2-way interaction analyses, 3-way interaction analyses were performed. This was to investigate whether fetal growth patterns, over time, of infants born LGA, versus infants born non-LGA, varied according to maternal BMI.

These analyses incorporated a three-way interaction between LGA status, time, and maternal BMI. Otherwise, these models were constructed the same as the 2-way interaction models. BMI was modelled as a continuous variable, but three different BMI values (22.0, 27.0 and 35.0 kg/m^2) were used for deriving estimates. There are two sets of interaction p values reported: the first is a test of 3-way interaction. The second set consists of the three tests of LGA-by-time interaction at each level of BMI (that is, at BMI 22.0 kg/m^2, does the difference between LGA and non-LGA vary over time; similarly, at BMI 27.0 kg/m^2, and at BMI 32.0 kg/m^2, does the difference vary over time).

5.5 Results

5.5.1 Baseline characteristics

Baseline characteristics of women included in these analyses are presented in Table 5.1. The overall mean BMI at trial entry was 30.71 kg/m^2 (SD 6.92 kg/m^2). The majority of women were in their second or subsequent pregnancy, were non-smokers, and were of Caucasian ethnicity. More than half of the women were from the two most socioeconomically disadvantaged quintiles of the Index of Relative Socioeconomic Disadvantage[243]. These baseline demographics are similar to those of the three randomised trials from which the women were recruited[188, 201, 204]. There were 573 LGA infants (17.58%) included.

Table 5.1 – Participant baseline characteristics: LGA vs. non-LGA infant fetal growth and adiposity

Characteristic	Overall Cohort*
Overall numbers	3260
BMI (kg/m²): Mean (SD)	30.71 (6.92)
BMI Category (kg/m²): N(%)	
18.5-24.9	628 (19.26)
25.0-29.9	1064 (32.64)
30.0-34.9	772 (23.68)
35.0-39.9	478 (14.66)
≥40.0	318 (9.75)
Parity: N(%)	
0	1411 (43.28)
1+	1849 (56.72)
Smoking status: N(%)	
Non-smoker	2848 (87.36)
Smoker	363 (11.13)
Missing	49 (1.50)
Quintile of Relative Socioeconomic Disadvantage: N(%)	
Q1 (most disadvantaged)	913 (28.01)
Q2	836 (25.64)
Q3	480 (14.72)
Q4	565 (17.33)
Q5 (least disadvantaged)	464 (14.23)
Ethnicity: N(%)	
Caucasian	2770 (84.97)
Non-Caucasian	490 (15.03)
LGA: N(%)	573 (17.58)

*The overall cohort includes all women who had one or more ultrasound scans during pregnancy in the LIMIT, GRoW and Optimise randomised trials, from both Standard Care and intervention groups.
BMI, body mass index. SD, standard deviation. N, number. IRSD, Index of Relative Socioeconomic Disadvantage[243]. LGA, large for gestational age.

5.5.2 Fetal growth measures of LGA vs. non-LGA infants

Results of the 2-way interaction of LGA and timing of ultrasound analysis are presented in Table 5.2. Infants born LGA, in comparison with those born non-LGA, were larger in all fetal growth measures, at all time points assessed, and these differences increased over time. The greatest differences in fetal biometry measures between infants born LGA, and those born non-LGA, were seen for fetal AC measures at all time points (Table 5.2). Calculated EFW, which is a function of the fetal biometry measures of BPD, HC, FL, and AC[244], similarly was greater among infants born LGA, compared with those born non-LGA, at both 28 and 36 weeks' gestation.

Table 5.2 – Fetal biometry of LGA vs. non-LGA infants

Outcome	LGA Mean (SD)	Non-LGA Mean (SD)	Estimate Mean difference (95% CI)	P value
BPD (cm)				<0.001*
20 weeks	4.67 (0.34)	4.60 (0.33)	0.06 (0.03, 0.09)	<0.001
28 weeks	7.35 (0.43)	7.18 (0.42)	0.16 (0.12, 0.20)	<0.001
36 weeks	9.15 (0.35)	8.87 (0.39)	0.27 (0.23, 0.30)	<0.001
HC (cm)				<0.001*
20 weeks	17.38 (1.17)	17.09 (1.13)	0.25 (0.15, 0.36)	<0.001
28 weeks	27.02 (1.39)	26.43 (1.33)	0.55 (0.41, 0.68)	<0.001
36 weeks	32.84 (1.09)	32.02 (1.22)	0.78 (0.67, 0.89)	<0.001
FL (cm)				<0.001*
20 weeks	3.26 (0.28)	3.19 (0.29)	0.06 (0.03, 0.08)	<0.001
28 weeks	5.40 (0.34)	5.28 (0.32)	0.11 (0.08, 0.14)	<0.001
36 weeks	7.05 (0.30)	6.85 (0.32)	0.19 (0.16, 0.22)	<0.001
AC (cm)				<0.001*
20 weeks	15.57 (1.28)	15.14 (1.23)	0.35 (0.23, 0.47)	<0.001
28 weeks	25.49 (1.72)	24.36 (1.58)	1.05 (0.88, 1.21)	<0.001
36 weeks	34.39 (1.91)	32.28 (1.70)	2.02 (1.83, 2.20)	<0.001
EFW (g)				<0.001*
28 weeks	1390.21 (253.48)	1248.15 (215.33)	131.99 (107.78, 156.20)	<0.001
36 weeks	3258.66 (399.93)	2813.16 (355.66)	435.44 (396.66, 474.22)	<0.001

LGA, large for gestational age. CI, confidence interval. SD, standard deviation. BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. * denotes the p value for the interaction term.

5.5.3 Fetal growth measure z-scores of LGA vs. non-LGA infants

In keeping with the above, fetal BPD, HC, FL, AC, and EFW z-scores were significantly greater among infants born LGA, compared with those born non-LGA, at all time points assessed, and these differences increased over time (Table 5.3).

With the exception of fetal BPD z-score at 36 weeks' gestation among non-LGA infants (-0.04 (SD 1.15) cm), all z-scores were positive at all time points, indicating that, even among those infants not born LGA, fetuses were larger, on average, than the reference population, likely because of the comparatively disproportionate number of women included in this cohort who were overweight or obese (Table 5.1).

Table 5.3 – Fetal biometry z-scores of LGA vs. non-LGA infants

Outcome	LGA Mean (SD)	Non-LGA Mean (SD)	Estimate Mean difference (95% CI)	P value
BPD z-score				<0.001*
20 weeks	0.40 (1.03)	0.12 (1.02)	0.26 (0.17, 0.36)	<0.001
28 weeks	1.19 (1.44)	0.46 (1.47)	0.71 (0.57, 0.85)	<0.001
36 weeks	0.82 (1.06)	-0.04 (1.15)	0.84 (0.74, 0.95)	<0.001
HC z-score				<0.001*
20 weeks	0.93 (0.73)	0.67 (0.71)	0.25 (0.18, 0.31)	<0.001
28 weeks	1.23 (0.95)	0.70 (0.90)	0.51 (0.42, 0.61)	<0.001
36 weeks	1.20 (0.78)	0.57 (0.86)	0.62 (0.54, 0.70)	<0.001
FL z-score				<0.001*
20 weeks	0.59 (0.66)	0.35 (0.71)	0.22 (0.15, 0.28)	<0.001
28 weeks	0.63 (1.03)	0.15 (0.94)	0.45 (0.35, 0.54)	<0.001
36 weeks	0.80 (0.97)	0.06 (0.99)	0.71 (0.62, 0.81)	<0.001
AC z-score				<0.001*
20 weeks	1.29 (1.03)	0.79 (1.03)	0.46 (0.36, 0.55)	<0.001
28 weeks	1.21 (0.98)	0.35 (0.88)	0.83 (0.73, 0.92)	<0.001
36 weeks	1.50 (1.02)	0.23 (0.87)	1.22 (1.12, 1.31)	<0.001
EFW z-score				<0.001*
28 weeks	0.95 (1.00)	0.09 (0.78)	0.82 (0.72, 0.91)	<0.001
36 weeks	1.31 (0.92)	0.07 (0.77)	1.19 (1.11, 1.28)	<0.001

LGA, large for gestational age. CI, confidence interval. SD, standard deviation. BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. * denotes the p value for the interaction term.

5.5.4 Fetal adiposity measures of LGA vs. non-LGA infants

With the exception of AFM measures at 28 weeks' gestation, all adiposity measures were greater among infants born LGA, compared with those born non-LGA, at all time points, and these differences increased over time (Table 5.4). The magnitude of these estimated mean differences ranged from 0.12 mm (95% CI 0.00, 0.24) for SSFM measurements at 28 weeks' gestation, up to 2.01 cm^2 (95% CI 1.53, 2.48) for MTLM measurements at 36 weeks' gestation (Table 5.4).

Table 5.4 – Fetal adiposity measures of LGA vs. non-LGA infants

Measure	LGA Mean (SD)	Non-LGA Mean (SD)	Estimated Mean difference (95% CI)	P value
MTLM (cm^2)				0.003*
28 weeks	5.34 (1.07)	4.82 (1.04)	0.50 (0.36, 0.64)	<0.001
36 weeks	9.79 (2.07)	8.80 (1.93)	0.98 (0.68, 1.27)	<0.001
MTFM (cm^2)				<0.001*
28 weeks	5.06 (1.35)	4.46 (1.19)	0.58 (0.41, 0.75)	<0.001
36 weeks	12.76 (3.42)	10.74 (2.74)	2.01 (1.53, 2.48)	<0.001
AFM (mm)				<0.001*
28 weeks	3.67 (0.95)	3.54 (1.00)	0.08 (-0.05, 0.21)	0.229
36 weeks	6.32 (1.72)	5.46 (1.58)	0.80 (0.57, 1.04)	<0.001
SSFM (mm)				<0.001*
28 weeks	3.36 (0.95)	3.16 (0.85)	0.12 (0.00, 0.24)	0.047
36 weeks	5.55 (1.60)	4.89 (1.38)	0.59 (0.38, 0.79)	<0.001

MTLM, mid-thigh lean mass. MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. LGA, large for gestational age. SD, standard deviation. CI, confidence interval. * denotes the p value for the interaction term.

5.5.5 Effect of maternal BMI - Fetal biometry measures and fetal biometry measure z-scores

There was no evidence that the relationships described in 5.5.2 and 5.5.3 differed by maternal BMI (data not shown).

5.5.6 Effect of maternal BMI - Fetal adiposity measures

There was no evidence that the relationships described in 5.5.4 differed by maternal BMI (data not shown).

5.6 Discussion

The analyses presented suggest that infants born LGA have larger fetal biometry measures, and higher growth trajectories, evident from 20 weeks' gestation. Similarly, fetal adiposity measures are consistently larger among infants born LGA and these differences increase over time. There was no evidence that the differences in biometry and adiposity measurements, including changes in magnitude over time, varied according to maternal BMI. These findings add to the growing body of evidence that fetal growth and growth trajectories are "set" and that infants born LGA exhibit differences from early in pregnancy.

Previous work investigating altered fetal growth and fetal growth trajectories among infants born LGA have focused on fetal AC measurements. Wong *et al* demonstrated that fetal AC z-scores of infants born LGA were larger on average than those of infants not born LGA, evident from as early as 18 weeks' gestation[245]. Madendag *et al* showed that infants born LGA had greater mean AC, and thus EFW, measurements at 26-28 weeks' gestation[132]. Similarly, Caradeux *et al* have shown both fetal AC z-score and AC z-score velocity are predictive of risk of birth of LGA infants[133]. Higher fetal growth rate between the first and second trimester has been associated with an increased risk of infant birth weight greater than 4500gm, or greater than 2 standard deviations above the mean[246]. Taken together, these findings suggest differences in fetal growth may be evident from early in the first trimester of pregnancy.

In comparison to this previous work, however, the analyses presented here considered all fetal biometry measures, and have shown significant differences in all aspects of fetal growth from as early as 20 weeks' gestation. Taken together, this suggests that fetal skeletal, organ, and adipose tissue growth are all impacted by factors contributing to LGA.

These analyses demonstrate that all fetal adiposity measures performed were greater among infants born LGA from early in pregnancy, and that this difference increased with time. These data represent the largest cohort of women who have

had longitudinal ultrasound scans assessing fetal adiposity measures. Interestingly, these results are in agreement with other published cohorts. Among a group of 702 Chinese women who had fetal biometry and adiposity measures performed at 28 and 36 weeks' gestation, population and ethnicity-specific reference ranges were defined for AFM and SSFM measures[247]. In comparison with this Chinese cohort, the mean AFM measurements among both LGA and non-LGA infants in these analyses were significantly larger[247]. There were differences in mean SSFM measurements between the data presented here and those presented by Chen *et al*, with infants born LGA having generally larger SSFM measurements in comparison[247]. There are some important differences between the population of women undergoing research ultrasounds in the analyses presented in this chapter and the women recruited to the study by Chen[247] that may account for some of these differences. In particular, the current analyses included predominantly Caucasian women (n=2,770; 84.97%), with the mean maternal BMI 30.71 kg/m^2. In comparison, the population described by Chen consisted of Asian women, all with a normal BMI (18.5-24.9 kg/m^2)[247].

With regards to fetal subcutaneous tissue thickness measurements, most interest in the literature has focused on AFM, and the utility of these measurements in predicting birthweight and risk of LGA infants[121, 248-250]. Abdominal fat mass measures taken during the third trimester have variably been associated with birthweight and neonatal adiposity measures[127, 129, 251], suggesting that fetal AFM measurements may not be the most reliable fetal subcutaneous tissue measurement to identify the fetus at risk of being born LGA. Additional investigations of other fetal subcutaneous tissue measurements, and overall fetal body composition, are required.

Being born LGA represents an independent risk factor for childhood obesity[252], and may lie on the causal pathway of the intergenerational cycle of obesity[253]. There has been significant interest in the literature on prevention of LGA birth by antenatal interventions, usually commenced after the first trimester of pregnancy[188, 193, 201, 220, 254]. The findings of this current study, which indicate

that accelerated growth of infants born LGA occur as early as 20 weeks' gestation, provides insight into why antenatal interventions have thus far been ineffective. That is, antenatal interventions commencing around or after 20 weeks' gestation may commence after these aberrant growth patterns are "set". Future chapters will consider antenatal interventions for prevention of LGA, and improvement of perinatal outcomes among women who are overweight or obese.

Chapter 6: effect of an antenatal diet and lifestyle intervention on fetal growth and adiposity among women who are of a normal BMI

6.1 Background

The Optimise trial evaluated an antenatal diet and lifestyle intervention, compared to standard care, among women of a normal BMI in early pregnancy[201]. In this cohort, exposure to an antenatal diet and lifestyle intervention was associated with an improvement in maternal diet quality, but there was no difference in risk of birth of a LGA infant, or birthweight >4kg[201]. However, whether an antenatal diet and lifestyle intervention affects fetal growth and adiposity in this cohort is not known.

What is known?
Among women of a normal BMI in early pregnancy, an antenatal diet and lifestyle intervention is not associated with differences in rates of birth of a LGA infant.
What is not known?
Whether an antenatal diet and lifestyle intervention is associated with alterations in fetal adiposity and body composition among women who are of a normal BMI in early pregnancy.
What do these analyses add to previous knowledge?
Among women who are of a normal BMI, there was no evidence that an antenatal diet and lifestyle intervention altered fetal biometry or adiposity measures, or their velocities over the third trimester.

6.2 Aim

To assess the effects of an antenatal dietary and lifestyle on fetal growth and adiposity among women who were of a normal BMI in early pregnancy

6.3 Objectives

The objectives of this chapter are to compare the effect of an antenatal dietary and lifestyle intervention to standard care, among women of a normal BMI in early pregnancy, on:

- fetal biometry measures
- fetal biometry measure velocity
- fetal adiposity measures in the third trimester
- fetal adiposity measure velocity

6.4 Methods pertaining to this chapter

6.4.1 Description of the cohort

The analyses reported in this chapter use data obtained from research ultrasounds of participants recruited to the Optimise randomised trial[201, 207], and randomised to either the Standard Care group or the Lifestyle Advice group. Details regarding the intervention and antenatal care have been discussed previously (Chapter 2 – Methods).

6.4.2 Detectable difference calculation

There were 633 participants with fetal ultrasound data for at least one time point. Calculations were performed using only the number of participants with ultrasounds at both 28 and 36 weeks, which was 257 in each group. Additionally, a conservative correlation between repeated measures of 0.7 was assumed. These values were used as inputs to the *power repeated* function in Stata v16 (StataCorp, TX) to estimate detectable effect size, and showed that a total of 390 participants provide 80% power, with two-sided alpha 0.05, to detect differences between the intervention groups of 0.14 SD.

6.4.3 Statistical analysis

The effect of the diet and lifestyle intervention, compared to standard care, on fetal growth outcomes, and fetal growth velocity outcomes, was assessed using linear regression models. Generalised Estimating Equations (GEE) were used to account for correlation due to repeated measures, and a timepoint-by-intervention interaction term to allow for differences in intervention effect between time points. The effect of the intervention, as the difference in means (Lifestyle Advice – Standard Care) and 95% Confidence Interval, was estimated at each time point. Biometry and adiposity measure velocities between 28 and 36 weeks did not include time-by-intervention interaction terms or GEEs, as these are not repeated measures. Analyses were adjusted for parity (0 vs. 1+), maternal prepregnancy BMI (as a continuous variable), maternal age at randomisation, smoking status, and SEIFA IRSD quintile as covariates. In addition, models for fetal growth outcomes other than z-scores included adjustment for the actual gestational age at ultrasound.

6.5 Results

6.5.1 Baseline characteristics

These analyses pertain to the 633 women recruited and randomised to the Optimise randomised trial who attended for at least one research ultrasound over the course of their pregnancy (317 in the Lifestyle Advice group, and 316 in the Standard Care group) (Figure 2.3). Baseline demographic characteristics of participating women who underwent at least one research ultrasound over pregnancy are shown in Table 6.1, and were comparable between the two groups. The median BMI in the cohort was 22.20 kg/m^2 (IQR 20.87 – 23.60 kg/m^2). The majority of women were in their first ongoing pregnancy, were non-smokers, and of Caucasian ethnicity.

Table 6.1 – Baseline characteristics: participants in the Optimise randomised trial who attended for one or more research ultrasounds

Characteristic	Lifestyle Advice Group	Standard Care Group	Total Cohort
Overall numbers	317	316	633
BMI (kg/m²): Median (IQR)	22.17 (20.81, 23.70)	22.20 (20.90, 23.46)	22.20 (20.87, 23.60)
Parity: N(%)			
0	189 (59.81)	186 (58.68)	375 (59.24)
1+	128 (40.19)	130 (41.32)	258 (40.76)
Smoking Status: N(%)			
Non-smoker	301 (95.25)	302 (95.27)	603 (95.26)
Smoker	15 (4.75)	13 (4.10)	28 (4.42)
Missing	0 (0.00)	2 (0.63)	2 (0.32)
Quintile of Relative Socioeconomic Disadvantage: N(%)			
Q1 (most disadvantaged)	48 (15.19)	58 (18.30)	106 (16.75)
Q2	78 (24.68)	93 (29.34)	171 (27.01)
Q3	48 (15.19)	39 (12.30	87 (13.74)
Q4	80 (25.32)	76 (23.97)	156 (24.64)
Q5 (least disadvantaged)	62 (19.62)	51 (16.09)	113 (17.85)
Ethnicity: N(%)			
Caucasian	212 (67.09)	215 (67.82)	427 (67.46)
Non-Caucasian	105 (32.91)	101 (32.18)	206 (32.54)

SD, standard deviation. IQR, interquartile range. N, number, Q, quintile.

6.5.2 Effect of an antenatal diet and lifestyle intervention on fetal biometry measures, among women of a normal BMI

Among women who were of a normal BMI in early pregnancy, there was no significant effect of the antenatal intervention on fetal biometry measures, or calculated EFW, at any of the time points assessed (Table 6.2). The estimates of effect size for fetal biometry measures were all less than 0.30 cm and crossed zero (Table 6.2), suggesting that the true difference in average fetal biometry measures between the Lifestyle Advice and Standard Care groups was small in absolute terms, and that the true effect of treatment is therefore unlikely to be clinically relevant.

Table 6.2 – Effect of intervention on ultrasound measures of fetal biometry among women who a normal BMI

Outcome	Lifestyle Advice Group Mean (SD)	Standard Care Group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD (cm)				0.726*		0.229*
20 weeks	4.55 (0.26)	4.56 (0.28)	-0.01 (-0.05, 0.03)	0.593	-0.01 (-0.04, 0.02)	0.555
28 weeks	7.17 (0.42)	7.16 (0.42)	0.01 (-0.05, 0.08)	0.685	0.02 (-0.03, 0.07)	0.385
36 weeks	8.85 (0.39)	8.86 (0.44)	-0.01 (-0.08, 0.06)	0.774	-0.01 (-0.08, 0.05)	0.694
HC (cm)				0.781*		0.729*
20 weeks	16.90 (0.84)	16.89 (0.87)	0.00 (-0.13, 0.14)	0.986	0.01 (-0.09, 0.11)	0.842
28 weeks	26.56 (1.32)	26.54 (1.28)	0.04 (-0.18, 0.25)	0.740	0.06 (-0.09, 0.21)	0.400
36 weeks	32.24 (1.17)	32.15 (1.25)	0.08 (-0.12, 0.28)	0.438	0.06 (-0.12, 0.24)	0.507
FL (cm)				0.598*		0.514*
20 weeks	3.10 (0.21)	3.11 (0.23)	-0.01 (-0.04, 0.03)	0.626	-0.01 (-0.03, 0.02)	0.573
28 weeks	5.28 (0.33)	5.28 (0.32)	-0.00 (-0.05, 0.05)	0.995	0.01 (-0.03, 0.04)	0.642
36 weeks	6.91 (0.29)	6.89 (0.33)	0.02 (-0.03, 0.07)	0.460	0.01 (-0.03, 0.05)	0.518

AC (cm)				0.820*		0.959*
20 weeks	14.96 (0.94)	14.93 (0.95)	0.02 (-0.13, 0.17)	0.801	0.03 (-0.08, 0.14)	0.623
28 weeks	24.37 (1.40)	24.39 (1.43)	-0.02 (-0.25, 0.21)	0.854	0.01 (-0.15, 0.17)	0.888
36 weeks	32.26 (1.71)	32.17 (1.71)	0.07 (-0.21, 0.35)	0.625	0.03 (-0.19, 0.26)	0.771
EFW (g)				0.588*		0.723*
28 weeks	1248.45 (201.89)	1250.03 (204.74)	-1.41 (-34.90, 32.08)	0.934	5.67 (-14.54, 25.88)	0.583
36 weeks	2833.38 (353.04)	2815.63 (378.80)	14.60 (-46.21, 75.42)	0.638	12.28 (-33.49, 58.05)	0.599

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW estimated fetal weight. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj treatment effect, adjusted treatment effect. Adj p-value, adjusted p value. SD, standard deviation. Adjusted models include parity (O vs. 1+), prepregnancy BMI (as a continuous variable), maternal age at randomization (as a continuous variable), smoking status, SEIFA IRSD quintile, and actual gestational age at ultrasound as covariates. * denotes the p value for the test that the interaction term = 0.

6.5.3 Effect of an antenatal diet and lifestyle intervention on fetal biometry measure z-scores

There were no statistically significant differences in the fetal biometry measure or calculated EFW z-scores between the treatment groups, at any of the time points assessed (Table 6.3). There was no evidence that the treatment effect differed between time points. All fetal biometry measure, and calculated EFW, z-scores were positive, suggesting the fetuses in this cohort were larger, on average, than the reference population used.

Table 6.3 – Effect of intervention on fetal biometry z-scores among women with a normal BMI

Outcome	Lifestyle Advice group Mean (SD)	Control group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD z-score				0.161*		0.174*
20 weeks	0.10 (0.86)	0.16 (0.99)	-0.06 (-0.21, 0.08)	0.383	-0.06 (-0.20, 0.09)	0.446
28 weeks	0.54 (1.49)	0.43 (1.53)	0.12 (-0.12, 0.37)	0.324	0.13 (-0.12, 0.37)	0.303
36 weeks	0.10 (0.86)	0.16 (0.99)	-0.06 (-0.21, 0.08)	0.383	-0.06 (-0.20, 0.09)	0.446
HC z-score				0.529*		0.580*
20 weeks	0.68 (0.58)	0.67 (0.61)	0.00 (-0.09, 0.10)	0.949	0.01 (-0.08, 0.10)	0.820
28 weeks	0.90 (0.88)	0.83 (0.88)	0.08 (-0.06, 0.22)	0.270	0.08 (-0.06, 0.22)	0.260
36 weeks	0.71 (0.82)	0.66 (0.90)	0.05 (-0.09, 0.19)	0.495	0.05 (-0.09, 0.19)	0.458
FL z-score				0.353*		0.390*
20 weeks	0.21 (0.59)	0.23 (0.59)	-0.02 (-0.11, 0.07)	0.709	-0.01 (-0.11, 0.08)	0.789
28 weeks	0.24 (0.87)	0.18 (0.93)	0.07 (-0.07, 0.22)	0.330	0.07 (-0.07, 0.22)	0.316
36 weeks	0.24 (0.87)	0.19 (0.94)	0.06 (-0.09, 0.21)	0.448	0.06 (-0.09, 0.20)	0.455

AC z-score				0.961*		0.951*
20 weeks	0.88 (0.95)	0.86 (0.94)	0.02 (-0.13, 0.17)	0.760	0.03 (-0.12, 0.18)	0.680
28 weeks	0.41 (0.81)	0.41 (0.77)	0.00 (-0.13, 0.13)	0.979	0.01 (-0.12, 0.13)	0.921
36 weeks	0.16 (0.85)	0.14 (0.86)	0.01 (-0.13, 0.15)	0.882	0.01 (-0.13, 0.15)	0.875
EFW z-score				0.833*		0.872*
28 weeks	0.18 (0.76)	0.15 (0.75)	0.03 (-0.09, 0.16)	0.584	0.03 (-0.09, 0.16)	0.579
36 weeks	0.08 (0.76)	0.05 (0.83)	0.02 (-0.11, 0.15)	0.723	0.03 (-0.10, 0.15)	0.683

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW estimated fetal weight. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj treatment effect, adjusted treatment effect. Adj p-value, adjusted p value. SD, standard deviation. Adjusted models include parity (O vs. 1+), prepregnancy BMI (as a continuous variable), maternal age at randomization (as a continuous variable), smoking status and SEIFA IRSD quintile as covariates. * denotes the p value for the test that the interaction term = 0.

6.5.4 Effect of an antenatal diet and lifestyle intervention on fetal adiposity measures, among women of a normal BMI

There was no statistically significant treatment effect of Lifestyle Advice, compared to Standard Care, on any of the measures of fetal adiposity, at either 28 or 36 weeks' gestation (Table 6.4). Estimates of effect size for all adiposity measures were close to zero, and the range of the 95% confidence intervals were small, suggesting a clinically meaningful effect is unlikely.

Table 6.4 – Effect of intervention on ultrasound fetal adiposity measures among women with a normal BMI

Outcome	Lifestyle Advice group Mean (SD)	Standard Care group Mean (SD)	Unadjusted treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
MTFM (cm^2)				0.905*		0.928*
28 weeks	4.47 (1.22)	4.47 (1.18)	-0.02 (-0.28, 0.24)	0.874	-0.06 (-0.29, 0.18)	0.631
36 weeks	10.63 (2.65)	10.66 (2.69)	0.02 (-0.61, 0.64)	0.956	-0.03 (-0.63, 0.57)	0.922
AFM (mm)				0.664*		0.552*
28 weeks	3.51 (0.88)	3.52 (0.93)	-0.01 (-0.19, 0.18)	0.946	-0.02 (-0.20, 0.17)	0.847
36 weeks	5.20 (1.68)	5.09 (1.62)	0.07 (-0.29, 0.44)	0.691	0.09 (-0.27, 0.45)	0.615
SSFM (mm)				0.201*		0.263*
28 weeks	3.13 (0.70)	3.11 (0.73)	0.02 (-0.13, 0.17)	0.786	0.01 (-0.14, 0.16)	0.890
36 weeks	4.09 (1.23)	4.25 (1.31)	-0.16 (-0.43, 0.10)	0.224	-0.15 (-0.42, 0.11)	0.263

MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. SD, standard deviation. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj treatment effect, adjusted treatment effect. Adj p-value, adjusted p value. SD, standard deviation. * denotes the p value for the interaction term. Adjusted models include parity (0 vs. 1+), prepregnancy BMI (continuous), maternal age at randomization (continuous), smoking status, SEIFA IRSD quintile, and actual GA at ultrasound as covariates. * denotes the p value for the test that the interaction term = 0

6.5.5 Effect of antenatal diet and lifestyle intervention on fetal growth velocity, among women of a normal BMI

With regards to fetal biometry velocities, and estimated fetal weight velocity, there were no statistically significant differences by treatment group, either in the raw velocity measurements or velocity z-scores (Table 6.5). Again, estimates of effect size were small, and the confidence intervals crossed zero, making a true clinically relevant difference unlikely.

Table 6.5 – Effect of intervention on fetal biometry velocities among women with a normal BMI

Outcome	Lifestyle Advice group Mean (SD)	Standard Care group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD (cm/wk)	0.03 (0.01)	0.03 (0.01)	-0.00 (-0.00, 0.00)	0.151	-0.00 (-0.00, 0.00)	0.180
HC (cm/wk)	0.10 (0.02)	0.10 (0.02)	-0.00 (-0.01, 0.00)	0.309	-0.00 (-0.01, 0.00)	0.269
FL (cm/wk)	0.03 (0.00)	0.03 (0.00)	0.00 (-0.00, 0.00)	0.703	0.00 (-0.00, 0.00)	0.678
AA (cm^2/wk)	0.64 (0.10)	0.64 (0.10)	-0.00 (-0.02, 0.02)	0.995	0.00 (-0.02, 0.02)	0.891
EFW (g/wk)	28.49 (3.73)	28.50 (4.25)	-0.00 (-0.70, 0.69)	0.990	0.06 (-0.63, 0.75)	0.862
BPDv z-score	0.53 (0.99)	0.65 (1.02)	-0.13 (-0.30, 0.05)	0.153	-0.11 (-0.29, 0.06)	0.204
AAv z-score	0.24 (0.68)	0.25 (0.70)	-0.00 (-0.12, 0.12)	0.978	0.01 (-0.11, 0.13)	0.901
FLv z-score	0.67 (0.80)	0.63 (0.99)	0.05 (-0.11, 0.20)	0.558	0.05 (-0.11, 0.21)	0.524
EFWv z-score	0.39 (0.78)	0.40 (0.91)	-0.01 (-0.16, 0.14)	0.898	0.00 (-0.14, 0.15)	0.957

BPD, biparietal diameter. HC, head circumference. FL, femur length. AA, abdominal area. EFW, estimated fetal weight. BPD, biparietal diameter velocity. AAv, abdominal area velocity. FLv, femur length velocity. EFWv, estimated fetal weight velocity. SD, standard deviation. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj treatment effect, adjusted treatment effect. Adj p-value, adjusted p value. SD, standard deviation.

6.6 Discussion

These results demonstrate that, among pregnant women who have a normal BMI in early pregnancy, there was no significant effect of an antenatal diet and lifestyle intervention on fetal biometry and adiposity measures, or growth velocities in late pregnancy.

The cohort of women recruited to the Optimise trial represent a large, prospective cohort of women at low risk for adverse pregnancy outcome, in particular a low risk of giving birth to an infant LGA. As a result, fetal growth and growth trajectories would be expected to approximate "normal", or the mean for a given gestational age, compared to a reference population in women of a normal BMI at low risk of pregnancy complications. However, z-scores of fetal biometry measures were generally >0 in this cohort (Table 6.4), suggesting the fetuses were, on average, larger than the reference population used to generate the z-scores[255]. This may be a result of alterations in population "normal" fetal growth and biometry measures over time, with mean birthweight and rates of LGA birth increasing temporally[100, 101]. The reference ranges used to generate z-scores in these results are in common clinical use, however were constructed 25 years ago, using data from less than 400 women who were described as "predominantly middle-class white" women[255]. There has been debate as to whether these reference ranges are still appropriate for assessing fetal growth and fetal growth abnormalities in contemporary patient populations[256].

Alternative, contemporary fetal growth standards have been published, however are not in wide-spread clinical use[257, 258]. Papageorghiou and colleagues published prospectively gathered fetal growth standards, and estimated fetal weight, among an ethnically diverse cohort of 4,607 women[257, 259]. Mean maternal BMI in this cohort (23.3 kg/m^2)[257] was similar to that presented here. Mean fetal biometry measures and estimated fetal weights presented here are close to the 50th percentile, and for some measures, at some gestations, below the 50th percentile[257, 259].

The World Health Organisation (WHO) similarly performed a contemporary international prospective study to determine fetal growth standards[258]. Mean maternal BMI in this cohort (23.1 kg/m^2)[258] was similar to that presented here. Again, mean fetal biometry measures and estimate fetal weights presented here are close to the 50th percentile, and for some measures at some gestations, just below the 50th percentile[258]. This reinforces the external validity and generalisability of the results presented here.

With regards to fetal adiposity measures, Larciprete and colleagues reported 5th, 50th, and 95th percentiles for mid-thigh fat mass, mid-thigh lean mass, abdominal fat mass, and subscapular fat mass over pregnancy among 218 "healthy" pregnant women[119]. In comparison with the current cohort, mean maternal BMI was slightly higher among those "healthy" pregnant women (24.3 kg/m^2), and mean maternal age was lower (27.4 years)[119]. However, despite these baseline characteristic differences, all mean fetal adiposity measures reported here are similar to the 50th percentile measurements at 28 and 36 weeks' gestation reported by Larciprete and colleagues[119].

In comparison to the fetuses born to women who are overweight or obese from the LIMIT trial, z-scores of fetal biometry measures were closer to 0 in this cohort, although they were still generally >0 (Table 6.4). With the exception of abdominal fat mass measures at 28 weeks' gestation, mean fetal adiposity measures were generally smaller among this cohort of women with a normal BMI in early pregnancy, compared with women who were overweight or obese[118]. This is in keeping with the findings presented in Chapter 3, where a continuous effect of increasing maternal BMI on fetal biometry and adiposity measures, with no threshold effect (Table 3.3 and 3.5). This further emphasises the strong effect of increasing maternal BMI on fetal growth and adiposity.

These results are the first to investigate the effects of an antenatal diet and lifestyle intervention on fetal biometry and adiposity measures over pregnancy among women who are of a normal BMI. Previous randomised trials of antenatal

diet and lifestyle interventions that have included women of a normal BMI in early pregnancy have not assessed the effects on fetal growth[260-264]. These results demonstrate that, among women who are of a normal BMI in early pregnancy, there is no evidence of an effect of an antenatal diet and lifestyle intervention on fetal biometry or adiposity measures over pregnancy, compared to those receiving standard care. Taken together with the findings presented earlier in this thesis, that maternal overweight and obesity exerts a strong positive effect on fetal growth and adiposity from as early as 20 weeks' gestation, it is also possible that fetal growth is "set" from very early in pregnancy, and interventions to alter this may need to be commenced earlier in pregnancy, or prior to pregnancy, to be effective.

Chapter 7: The effect of metformin in addition to an antenatal diet and lifestyle intervention on fetal growth and adiposity: the GRoW randomised trial

The analyses reported in this chapter have been published in BMC Endocrine Disorders[265]. The published manuscript is included as appendix 5.

7.1 Background

Antenatal dietary and lifestyle interventions have not been shown to be effective in reducing the risk of adverse clinical pregnancy and birth outcomes[189, 191, 266], and in particular, have not reduced the risk of high infant birthweight[191]. Oral metformin, a commonly used hypoglycaemic agent, has been evaluated as a strategy to improve pregnancy outcomes for overweight and obese women, given similarities in the metabolic environment between both obesity and GDM. However, studies investigating the effects of antenatal metformin have not reported fetal growth and adiposity[192, 267].

What is known?
Increasing maternal BMI is associated with increasing fetal growth and adiposity. Antenatal diet and lifestyle interventions alone have not been demonstrated to be effective at reducing the risk of high infant birthweight.
What is not known?
The effect of adjuvant metformin, in addition to an antenatal diet and lifestyle intervention, on fetal growth and adiposity, and fetal growth trajectories.
What do these analyses add to previous knowledge?
The addition of metformin to dietary and lifestyle advice in pregnant women who are overweight or obese has no evidence of a clinically relevant effect on fetal biometry or adiposity measures, or their velocities in late pregnancy.

7.2 Aim

To investigate, among overweight or obese pregnant women, the effect of a combined antenatal dietary and lifestyle intervention, and metformin, on fetal growth and adiposity.

7.3 Objectives

The objectives of this chapter are to assess, among women who are overweight or obese in pregnancy, the effect of metformin, as an adjunct to an antenatal dietary and lifestyle intervention on:

- fetal biometry measures;
- fetal biometry measure velocities;
- fetal adiposity measures in the third trimester; and
- fetal adiposity measure velocities.

7.4 Methods pertaining to this chapter

7.4.1 Description of the cohort

The analyses reported in this chapter use data obtained from research ultrasounds of participants of the GRoW randomised trial[204], and randomised to one of two treatment groups - the Metformin group or the Placebo group. Details regarding the intervention and antenatal care have been discussed previously (Chapter 2 – Methods).

7.4.2 Detectable difference calculation

In total, 511 participants provided fetal ultrasound data for at least one time point. The *power repeated* function in Stata v16 (StataCorp, TX) was used to perform effect size estimations. To provide a conservative estimate of detectable effect size, calculations were performed using an assumption of 2 time points, and using only the number of participants with ultrasounds at both 28 and 36 weeks, thus a sample size of 195 per group was used. These calculations showed that 390 participants provide 80% power, with two-sided alpha 0.05, to detect differences between the intervention groups of 0.14 SD.

7.4.3 Statistical analysis

Baseline characteristics of women included in the analysis were compared descriptively between treatment groups (Metformin group versus Placebo group). Continuous variables were reported as means and standard deviations, or as medians and interquartile ranges if not normally distributed. Categorical variables were reported as frequencies and percentages.

Outcomes measured at multiple time points were analysed using linear regression models, with Generalised Estimating Equations to account for correlation due to repeated measures, and a time-by-treatment interaction term to test for differences in treatment effect between time points. Estimates are reported as differences in means (Metformin group – Placebo group) and 95% confidence intervals for each time point separately, regardless of the significance of the interaction term. Growth velocity outcomes were analysed using linear regression models, with estimates again reported as differences in means

(Metformin Group – Placebo Group) and 95% confidence intervals. Analyses were adjusted for stratification variables (study centre, BMI category and parity), maternal age at trial entry, smoking status, and SEIFA IRSD quintile as covariates.

7.5 Results

7.5.1 Baseline characteristics

The present study includes a total of 511 women who attended for one or more research ultrasounds; 255 women from the Metformin group and 256 women from the Placebo group (Figure 2.2). Baseline demographic characteristics of participating women are shown in Table 7.1, and were comparable between treatment groups. The median gestational age at trial entry was 16.29 weeks (Interquartile Range (IQR) 14.43 - 18.00 weeks). The median BMI of the cohort was 32.30 kg/m^2 (IQR 28.90 - 37.20 kg/m^2), with most women in their second or subsequent pregnancy, non-smokers, and 62.63% from the highest two quintiles of social disadvantage[243]. These characteristics are similar to the full randomised cohort[204].

Table 7.1 – Baseline characteristics: participants in the GRoW randomised trial who attended for one or more research ultrasounds

Characteristic	Metformin Group	Placebo Group	Total
Overall Numbers	255	256	511
BMI (kg/m²): Median (IQR)	32.40 (28.70, 37.57)	32.05 (29.10, 36.80)	32.30 (28.90, 37.20)
BMI category (kg/m²): N(%)			
25.0-29.9	83 (32.55)	83 (32.42)	166 (32.49)
30.0-34.9	74 (29.02)	83 (32.42)	157 (30.72)
35.0-39.9	58 (22.75)	48 (18.75)	106 (20.74)
≥40.0	40 (15.69)	42 (16.41)	82 (16.05)
Parity: N(%)			
0	88 (34.51)	91 (35.55)	179 (35.03)
1+	167 (65.49)	165 (64.45)	332 (64.97)
Smoking Status: N(%)			
Non-smoker	230 (90.20)	208 (81.25)	438 (85.71)
Smoker	24 (9.41)	43 (16.80)	67 (13.11)
Missing	1 (0.39)	5 (1.95)	6 (1.17)
Quintile of Relative Socioeconomic Disadvantage: N(%)			

Q1(most disadvantaged)	75 (29.41)	93 (36.33)	168 (32.88)
Q2	78 (30.59)	74 (28.91)	152 (29.75)
Q3	31 (12.16)	30 (11.72)	61 (11.94)
Q4	52 (20.39)	43 (16.80)	95 (18.59)
Q5(least disadvantaged)	19 (7.45)	16 (6.25)	35 (6.85)
Ethnicity: N(%)			
Caucasian	209 (81.96)	219 (85.55)	428 (83.76)
Non-Caucasian	46 (18.04)	37 (14.45)	83 (16.24)

SD, standard deviation. IQR, interquartile range. BMI, body mass index. N, number. ATSI, Aboriginal or Torres Strait Islander. SEIFA IRSD, socio-economic Indexes for Areas Index of Relative Socio-economic Disadvantage, Q, quintile.

7.5.2 Effect of adjuvant metformin on fetal biometry measures

There were no significant differences in fetal biometry measures of BPD, HC or AC at any of the time points assessed (Table 7.2). Although measurements of FL were not statistically significantly different at 20 or 28 weeks' gestation, average femur length was greater in the Metformin group at 36 weeks' gestation compared to the Placebo group (Table 7.2). However the magnitude of the difference was small – only 0.07 (95% CI: 0.01, 0.14) cm – and, while statistically significant (p=0.019), it is considered unlikely to be clinically relevant. There were no differences in calculated EFW at 28 or 36 weeks' gestation, by treatment group (Table 7.2). The estimates of effect size for fetal biometry measures were all less than 0.50 cm and the 95% confidence intervals crossed zero (Table 7.2), suggesting that the true effect of treatment is unlikely to be clinically relevant. Similarly, the estimated effect size for estimated fetal weight was only 17.61 (95% CI -57.67, 92.88) gm (Table 7.2), again suggesting that the true effect is not clinically relevant.

Table 7.2 – Effect of adjuvant antenatal metformin on ultrasound measures of fetal biometry

Outcome	Metformin group Mean (SD)	Control group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD (cm)				0.138*		0.102*
20 weeks	4.66 (0.34)	4.69 (0.35)	-0.03 (-0.09, 0.03)	0.328	-0.04 (-0.09, 0.02)	0.232
28 weeks	7.13 (0.50)	7.15 (0.41)	-0.02 (-0.10, 0.06)	0.645	-0.02 (-0.10, 0.07)	0.671
36 weeks	8.94 (0.42)	8.88 (0.39)	0.06 (-0.01, 0.14)	0.110	0.07 (-0.01, 0.14)	0.076
HC (cm)				0.299*		0.200*
20 weeks	17.38 (1.20)	17.45 (1.36)	-0.07 (-0.29, 0.16)	0.551	-0.09 (-0.31, 0.13)	0.431
28 weeks	26.46 (1.55)	26.43 (1.26)	0.04 (-0.22, 0.30)	0.744	0.05 (-0.22, 0.31)	0.727
36 weeks	32.36 (1.23)	32.20 (1.21)	0.17 (-0.05, 0.40)	0.134	0.19 (-0.04, 0.42)	0.100
FL (cm)				0.018*		0.012*
20 weeks	3.26 (0.30)	3.28 (0.30)	-0.03 (-0.08, 0.02)	0.267	-0.04 (-0.09, 0.01)	0.127
28 weeks	5.29 (0.37)	5.29 (0.30)	0.01 (-0.06, 0.07)	0.841	0.00 (-0.06, 0.07)	0.909
36 weeks	6.92 (0.32)	6.85 (0.33)	0.08 (0.01, 0.14)	0.015	0.07 (0.01, 0.14)	0.019
AC (cm)				0.992*		0.959
20 weeks	15.56 (1.37)	15.65 (1.29)	-0.09 (-0.32, 0.14)	0.457	-0.10 (-0.32, 0.12)	0.368
28 weeks	24.62 (1.75)	24.70 (1.56)	-0.07 (-0.37, 0.24)	0.669	-0.05 (-0.36, 0.26))	0.747
36 weeks	32.82 (1.87)	32.92 (1.99)	-0.07 (-0.43, 0.29)	0.690	-0.07 (-0.43, 0.29)	0.707

EFW (g)				0.720*		0.756*
28 weeks	1272.36 (248.33)	1268.81 (201.23)	5.05 (-36.72, 46.82)	0.813	5.40 (-36.93, 47.74)	0.803
36 weeks	2932.32 (399.97)	2914.27 (417.68)	18.98 (-56.99, 94.95)	0.624	17.61 (-57.67, 92.88)	0.647

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj, treatment effect, adjusted treatment effect. Adj p-value, adjusted p value.

7.5.3 Effect of adjuvant metformin on fetal biometry measure z-scores

There were no significant differences in the fetal biometry z-scores of BPD, HC, FL or AC between the treatment groups, at any of the time points assessed, or calculated estimated fetal weight z-score at 28 or 36 weeks' gestation (Table 7.3). All fetal biometry measure z-scores were positive in both groups, at all time points, suggesting the fetuses of women in this study were larger on average than that of the reference population[213, 268].

Table 7.3 – Effect of adjuvant antenatal metformin on fetal biometry z-scores

Outcome	Metformin group Mean (SD)	Control group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD z-score				0.138*		0.102*
28 weeks	0.41 (1.54)	0.56 (1.47)	-0.14 (-0.41, 0.14)	0.333	-0.13 (-0.41, 0.14)	0.335
36 weeks	0.18 (1.23)	0.10 (1.14)	0.04 (-0.18, 0.26)	0.697	0.06 (-0.16, 0.28)	0.564
HC z-score				0.788*		0.708*
28 weeks	0.82 (1.02)	0.83 (0.88)	0.02 (-0.15, 0.20)	0.817	0.02 (-0.15, 0.20)	0.801
36 weeks	0.85 (0.89)	0.79 (0.86)	0.05 (-0.12, 0.21)	0.583	0.06 (-0.11, 0.22)	0.493
FL z-score				0.036*		0.031*
28 weeks	0.30 (0.89)	0.37 (0.95)	-0.06 (-0.23, 0.11)	0.496	-0.09 (-0.26, 0.08)	0.316
36 weeks	0.36 (1.03)	0.21 (1.03)	0.14 (-0.05, 0.32)	0.161	0.12 (-0.07, 0.30)	0.230
AC z-score				0.743*		0.696*
28 weeks	0.61 (0.92)	0.75 (0.98)	-0.13 (-0.31, 0.04)	0.134	-0.12 (-0.30, 0.05)	0.165
36 weeks	0.56 (1.06)	0.73 (1.01)	-0.16 (-0.35, 0.03)	0.098	-0.16 (-0.35, 0.03)	0.104

EFW z-score				0.890*		0.901*
28 weeks	0.32 (0.85)	0.40 (0.89)	-0.07 (-0.23, 0.09)	0.399	-0.07 (-0.23, 0.09)	0.398
36 weeks	0.42 (0.97)	0.48 (0.93)	-0.06 (-0.23, 0.12)	0.513	-0.06 (-0.23, 0.11)	0.500

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj, treatment effect, adjusted treatment effect. Adj p-value, adjusted p value.

7.5.4 Effect of adjuvant metformin on fetal adiposity measures

There was no significant treatment effect on any of the measures of fetal adiposity, at either 28 or 36 weeks' gestation (Table 7.4). Estimates of effect size for all adiposity measures were close to zero, and the range of the 95% confidence intervals were small, suggesting that the true effect is not clinically meaningful.

Table 7.4 – Effect of adjuvant antenatal metformin on ultrasound fetal adiposity measures

Outcome	Metformin group Mean (SD)	Control group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
MTFM (cm^2)				0.845*		0.843*
28 weeks	4.24 (1.03)	4.21 (1.09)	0.04 (-0.27, 0.35)	0.805	0.02 (-0.29, 0.33)	0.902
36 weeks	9.87 (2.79)	9.95 (2.47)	-0.05 (-0.95, 0.84)	0.908	-0.07 (-0.94, 0.80)	0.870
AFM (mm)				0.144*		0.199*
28 weeks	3.60 (1.17)	3.60 (1.08)	0.01 (-0.31, 0.34)	0.935	0.06 (-0.27, 0.39)	0.718
36 weeks	6.42 (1.41)	5.98 (1.51)	0.42 (-0.05, 0.88)	0.077	0.42 (-0.05, 0.89)	0.079
SSFM (mm)				0.919*		0.655*
28 weeks	3.22 (0.80)	3.20 (0.88)	0.02 (-0.21, 0.25)	0.863	0.03 (-0.19, 0.25)	0.760
36 weeks	4.84 (1.44)	4.85 (1.48)	-0.00 (-0.42, 0.41)	0.985	-0.07 (-0.48, 0.34)	0.740

MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. Unadj treatment effect, unadjusted treatment effect. Unadj p value, unadjusted p value. Adj treatment effect, adjusted treatment effect. Adj p-value, adjusted p value. * denotes the p value for the interaction term.

7.5.5 Effect of adjuvant metformin on fetal growth velocity

With regards to fetal biometry velocities and estimated fetal weight velocity, there were no significant differences between treatment groups, either in the raw velocity measurements or velocity z-scores for any measurement (Table 7.5).

Table 7.5 – Effect of adjuvant antenatal metformin on fetal growth velocities

Outcome	Metformin group Mean (SD)	Control group Mean (SD)	Unadj treatment effect (95% CI)	Unadj P-value	Adj treatment effect (95% CI)	Adj P-value
BPD velocity (cm/wk)	0.22 (0.04)	0.22 (0.04)	0.000 (-0.008, 0.009)	0.967	-0.003 (-0.011, 0.006)	0.555
HC velocity (cm/wk)	0.11 (0.02)	0.11 (0.02)	-0.002 (-0.006, 0.002)	0.398	-0.003 (-0.007, 0.001)	0.150
FL velocity (cm/wk)	0.03 (0.00)	0.03 (0.01)	0.000 (-0.001, 0.001)	0.403	0.000 (-0.001, 0.001)	0.491
AA velocity (cm^2/wk)	0.67 (0.14)	0.70 (0.14)	-0.021 (-0.048, 0.005)	0.118	-0.021 (-0.049, 0.006)	0.127
EFW velocity (g/wk)	29.93 (5.20)	30.32 (5.32)	-0.384 (-1.412, 0.645)	0.465	-0.405 (-1.460, 0.650)	0.452
BPD velocity z-score	0.80 (1.08)	0.69 (1.08)	0.104 (-0.106, 0.314)	0.331	0.034 (-0.175, 0.242)	0.751
AA velocity z-score	0.47 (0.94)	0.60 (0.97)	-0.132 (-0.317, 0.054)	0.164	-0.133 (-0.322, 0.057)	0.170
FL velocity z-score	0.70 (0.98)	0.56 (1.12)	0.144 (-0.061, 0.349)	0.168	0.128 (-0.081, 0.337)	0.231
EFW velocity z-score	0.69 (1.10)	0.77 (1.14)	-0.080 (-0.298, 0.139)	0.476	-0.084 (-0.309, 0.140)	0.462

BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. AA, abdominal area. BPDv, biparietal diameter velocity. AAv, abdominal area velocity. FLv, femur length velocity. EFWv, estimated fetal weight velocity. Unadj treatment effect, unadjusted treatment effect. Unadj p-value, unadjusted p value. Adj, treatment effect, adjusted treatment effect. Adj p-value, adjusted p value.

7.6 Discussion

These findings demonstrate that, among pregnant women who are overweight or obese, antenatal treatment with oral metformin as an adjunct to dietary and lifestyle advice did not appreciably impact measures of fetal biometry or adiposity, or fetal biometry growth velocities over the third trimester of pregnancy.

Metformin has been used increasingly in the treatment of GDM. The Metformin in Gestational Diabetes (MiG) Trial confirmed the safety and efficacy of metformin use in women with GDM[192]. Oral metformin was not associated with an increased rate of the composite neonatal adverse outcome (comprising neonatal hypoglycaemia, respiratory distress, need for phototherapy, birth trauma, 5-minute Apgar <7, or preterm birth)[192]. Rowan *et al* also found no significant differences between metformin and insulin with regards to neonatal biometry, circumferences, or skin-fold thickness measurements[192].

Subsequent childhood follow up at two years of age, however, revealed selected differences with children exposed to metformin in pregnancy having statistically significantly greater upper arm circumference, subscapular and biceps skinfold thicknesses, and greater fat free mass measurements, in comparison with children exposed to insulin antenatally[269]. It was hypothesised that antenatal metformin contributed to fat being stored in subcutaneous sites, resulting in less ectopic or visceral fat[269]. However, these differences were no longer evident at 7-9 years of age follow-up[270], suggesting the possibility of a chance finding and limited clinical relevance. Women recruited to the MiG study had an average early pregnancy BMI of 32 kg/m^2[192], similar to women in the GROW randomised cohort[193]. In contrast to the MiG study, however, women recruited to the GRoW randomised trial commenced treatment much earlier in pregnancy, with an average gestational age at trial entry of 16 weeks[204]. Furthermore, the MiG trial did not report fetal measures of growth and adiposity.

The findings of the current analysis are in contrast to those reported in a secondary analysis of the PregMet randomised trial, investigating the effect of adjuvant antenatal metformin treatment among women with polycystic ovary syndrome (PCOS) on fetal and neonatal biometry[267]. In this study the fetuses of women exposed to antenatal metformin were found to have larger BPD measurements at 32 weeks' gestation, and greater HC measurements at birth[267]. However, the magnitude of these differences was small – on average, less than one centimetre. This study had fewer participants than the GRoW randomised trial, and the average BMI at trial entry was 29 kg/m^2, compared with 32 kg/m^2 for women in the GRoW randomised trial[193], which may have contributed to the variability between studies.

All women randomised to the GRoW trial were exposed to the same dietary and lifestyle intervention as was provided in the LIMIT randomised trial[188]. The LIMIT randomised trial identified that the dietary and lifestyle intervention was associated with greater fetal mean mid-thigh fat mass, and a significantly slower rate of subscapular adipose tissue deposition, with no difference in lean thigh mass or abdominal fat mass[118]. Of interest, results of fetal biometry z-scores, estimated fetal weights and subcutaneous fat measures obtained from fetuses of women randomised to the GRoW trial placebo group were similar to those found in fetuses randomised to the diet and lifestyle group of the LIMIT randomised trial[188], providing further support for the robustness of the measurements in these very similar populations.

Women recruited to the GRoW randomised trial were, on average, 16 weeks' gestation. Sovio and colleagues[182] demonstrated that the fetuses of women who were obese were more likely to have an abdominal circumference measurement greater than the 90th percentile which was already evident at 20 weeks' gestation. There is increasing evidence that the preconception period is of vital importance to healthy growth and development[271, 272]. It is possible that interventions, such as the diet and lifestyle intervention investigated here, need to be commenced prior to conception, to have an appreciable effect on fetal growth and adiposity.

Among pregnant women who are overweight or obese, antenatal treatment with oral metformin as an adjunct to dietary and lifestyle advice did not appreciably impact measures of fetal biometry or adiposity, or fetal biometry growth velocities over the third trimester of pregnancy. As all women participating in the GRoW randomised trial received an antenatal diet and lifestyle intervention, it possible that the pathways involved in fetal fat deposition have been "saturated", and cannot be further affected by the addition of metformin. It is also possible that the intervention is commenced too late for appreciable effects to be seen in this high risk cohort. Future work should consider the importance of the preconception period in mitigating the effects of maternal overweight and obesity on fetal growth and adiposity.

Chapter 8: Overall conclusions

The analyses presented in this thesis investigate the effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity; whether this is mediated by GDM diagnosis and treatment; how fetal growth and adiposity are altered among infants born LGA; and finally, the effect of antenatal interventions to limit gestational weight gain on fetal growth and adiposity. Overall, these analyses demonstrate that maternal BMI exerts a strong, continuous positive effect on fetal growth and adiposity, from as early as 20 weeks' gestation. Among women who are overweight or obese, there is no evidence that the effect of BMI >30.0, vs BMI 25.0-29.9 kg/m^2, is mediated via diagnosed and treated GDM. Infants born LGA demonstrate larger fetal biometry and adiposity measures from as early as 20 weeks' gestation. And finally, the antenatal interventions investigated in this thesis have not demonstrated an ability to alter fetal growth and adiposity, most likely because the interventions are commenced too late in pregnancy to be clinically effective.

Overall, the findings presented in this thesis suggest fetal growth patterns are determined early in pregnancy, and any antenatal interventions to prevent the effects of maternal overweight and obesity on fetal growth will need to be commenced earlier in pregnancy, or prior to conception, to be effective.

The effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity

• The relationship between maternal BMI and fetal growth and adiposity measures is linear in nature, with no threshold effect seen
• Increasing maternal BMI is associated with increasing
  ○ Fetal biometry measures and calculated EFW, and fetal biometry and EFW z-scores, as early as 20 weeks' gestation
  ○ Fetal biometry measure and calculated EFW velocity in late pregnancy
  ○ Measures of fetal adiposity at 36 weeks', but not 28 weeks' gestation

Mediation of fetal growth and adiposity by GDM among women who are overweight or obese
• Among pregnant women who are obese, compared to women who are overweight, there is no evidence that the effect of BMI on fetal biometry or adiposity measures is mediated via diagnosed and treated GDM
Exploring fetal growth and adiposity of LGA and non-LGA infants

• Infants born LGA
  ○ Have larger fetal biometry measures and higher fetal biometry velocities from as early as 20 weeks' gestation
  ○ Have larger fetal adiposity measures at 28 and 36 weeks' gestation, with the exception of AFM measurements at 28 weeks' gestation

Effect of antenatal dietary and lifestyle advice on fetal growth and adiposity among women who are of a normal BMI

• Among pregnant women who have a normal BMI, there is no evidence of effect of an antenatal diet and lifestyle advice intervention on
  ○ fetal biometry or fetal biometry velocities in the third trimester
  ○ fetal adiposity or the rate of fetal adipose tissue accumulation in the third trimester

Effect of antenatal adjuvant metformin on fetal growth and adiposity among women who are overweight or obese

• Among pregnant women who are overweight or obese, there is no evidence that antenatal treatment with oral metformin as an adjunct to dietary and lifestyle advice
  ○ Alters fetal biometry, or fetal biometry growth velocities, over the third trimester of pregnancy
  ○ Impacts measures of fetal adiposity over the third trimester of pregnancy

8.1 Strengths and limitations

When combined, the three harmonised randomised trials that contributed data to the work in this thesis[188, 193, 201] represent one of the largest longitudinal, prospectively recruited cohorts of pregnant women published. This enabled the robust examination of fetal growth and adiposity during pregnancy in women, across the BMI spectrum. In particular, the large number of women included who were overweight or obese is a significant strength. Previously published work has included relatively small numbers of women at the upper range of maternal BMI, limiting statistical power and reliable conclusions to be made about the effects of maternal overweight and obesity on fetal growth. While it is known that increasing maternal BMI is associated with reduced accuracy in fetal ultrasound measures, good inter-observer agreement in measurements among women in this cohort who were overweight or obese has been shown[118].

A limitation of the studies presented here is that they represent secondary analyses, albeit made up of outcomes that were specified prior to the analysis. Despite this, the large number of women contributing ultrasound data results in significant power to detect clinically important differences in fetal growth and adiposity measures, should they exist. The women recruited drew from populations of women booking their pregnancy care at three major public maternity hospitals in South Australia, contributing to the generalisability of these findings to the state and national populations of pregnant women.

However, women recruited and randomised to the studies were predominantly of Caucasian ethnicity, and over half of the women were from the highest two quintiles of socioeconomic disadvantage. This may not be truly representative of the demographics of the state and country, as a whole, and may somewhat limit the generalisability of these findings.

8.2 Implications for clinical practice

There are currently no evidence-based antenatal interventions recommended for the prevention of adverse maternal and infant outcomes among women who are overweight or obese. In particular, there are no antenatal interventions to prevent birth of LGA infants, either among women who are overweight or obese, or those of a normal BMI. The three harmonised studies[188, 193, 201] contributing data for this thesis did not report clinically significant effects of an antenatal diet and lifestyle intervention or adjuvant metformin on pregnancy outcomes, including LGA. By exploring in detail the fetal growth and adiposity of these infants, this thesis provides strong evidence that fetal growth patterns are likely "set" early in pregnancy and before antenatal interventions such as those described have commenced.

It is possible that the antenatal interventions presented in this thesis were commenced too late to be effective, and that interventions to impact fetal growth must be started earlier in pregnancy, or prior to pregnancy, to have an effect. The data presented in this thesis suggests that maternal BMI exerts an effect on fetal growth from as early as 20 weeks' gestation, however data from ultrasounds prior to this gestation were not part of these studies. However, given these data show such early effects on fetal growth, it is likely that interventions will need to be commenced prior to pregnancy to be effective.

8.3 Implications for future research

With increasing rates of maternal overweight and obesity worldwide, there is an urgent need for evidence-based interventions to improve outcomes among this group of women who are at an increased risk of adverse maternal and infant outcomes. While antenatal interventions have been shown to be generally ineffective, future research focused on prepregnancy interventions to improve outcomes is required. Additionally, further work investigating fetal growth earlier than 20 weeks' gestation is important, to accurately determine if and when fetal growth is altered. Investigation of which, if any, of the fetal subcutaneous tissue fat measures of adiposity accurately predict newborn adiposity are required, as well as childhood and adolescent follow up to determine whether fetuses identified as having increased adiposity are at risk of subsequent childhood and adolescent obesity. Childhood and adolescent follow up of the children born to women included in both prepregnancy and antenatal intervention trials are required, to better elucidate the determinants of the intergenerational cycle of obesity, and how to prevent it.

Appendix 1

VP 34.02: The effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity

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Introduction

Women who are overweight or obese in pregnancy are at an increased risk of delivering an infant with high birthweight, and increased fat mass. However, which fetal biometry measures differ, and when during pregnancy fetal growth diverges, is not known. The development of fetal subcutaneous fat measures over the third trimester, and the effect of increasing maternal BMI on these measures, is also not known.

Objective

To describe the effect of increasing maternal, BMI, across the BMI spectrum, on fetal growth and adiposity.

Methods

The analyses use data obtained from research ultrasounds of participants randomised to the Standard Care groups of the LIMIT[1] and Optimise[2] randomised trials. Women with a singleton pregnancy, between 10^{+0} and 20^{+0} weeks' gestation were recruited to one of these trials, and received pregnancy care and birth at maternity hospitals in Adelaide, South Australia. Women recruited and randomised to the Standard Care groups of these trials underwent routine antenatal care according to local hospital ands state guidelines. Women underwent ultrasound assessment at 28 and 36 weeks' gestation at which time standard biometry measures were obtained, in addition to an assessment of fetal body composition. Fetal body composition and adiposity was assessed via mid thigh lean (MTLM) and fat mass (MTFM), abdominal fat mass (AFM) and subscapular fat mass (SSFM). These measurements have been described and validated in previous studies[6,7,8,9]. Analyses were performed using linear regression models, with GEEs for repeated measures. Models were adjusted for maternal age, parity, SEIFA IRSD Quintile, and smoking status. Results are presented as estimated difference in mean fetal measures corresponding to a 5 kg/m^2 increase in maternal BMI, and 95% confidence interval.

Results

Baseline characteristics

The cohort consisted of 1,425 women. Baseline characteristics were similar to the full randomised cohorts. Maternal BMI, meant weight and height at trial entry differed between the two groups, as a result of different inclusion criteria (Table 1). Women in the standard care group of the LIMIT trial were more likely to be multiparous and of Caucasian ethnicity.

Characteristics	LIMIT Randomised Trial	Optimise Randomised Trial
Overall numbers	1,060	317
BMI (kg/m^2): Mean (SD)	32.40 (5.94)	22.15 (1.66)
BMI category: N(%) 18.5-24.9 25.0-29.9 30.0-34.9 35.0-39.9 40.0+	 0 (0.00) 457 (43.11) 306 (28.87) 175 (16.51) 122 (11.51)	 317 (100.00) 0 (0.00) 0 (0.00) 0 (0.00) 0 (0.00)
Weight (kg) at trial entry: Mean (SD)	88.08 (17.58)	60.22 (6.85)
Height (cm) at trial entry: Mean (SD)	164.75 (6.46)	164.74 (7.17)
Parity: N(%) 0 1+	 425 (40.09) 635 (59.91)	 186 (58.68) 131 (41.32)
Smoking status: N(%) Nonsmoker Smoker Missing	 913 (86.13) 122 (11.51) 25 (2.36)	 302 (95.27) 13 (4.10) 2 (0.63)
Ethnicity: N(%) Caucasian Non-Caucasian	 962 (90.75) 98 (9.25)	 215 (67.82) 102 (32.18)

Table 1. Baseline characteristics of women who underwent one or more fetal ultrasound assessment

Outcome	Adjusted estimate (95% CI)	P value
BPD (cm) - 20 weeks - 28 weeks - 36 weeks	 0,04 (0,02, 0,05) 0,02 (0,00, 0,04) 0,03 (0,01, 0,04)	0,242* <0,001 0,049 0,001
HC (cm) - 20 weeks - 28 weeks - 36 weeks	 0,16 (0,11, 0,21) 0,07 (-0,00, 0,13) 0,08 (0,03, 0,14)	0,013* <0,001 0,052 0,004
FL (cm) - 20 weeks - 28 weeks - 36 weeks	 0,04 (0,03, 0,05) 0,01 (-0,00, 0,03) 0,01 (-0,01, 0,03)	0,001* <0,001 0,106 0,271
AC (cm) - 20 weeks - 28 weeks - 36 weeks	 0,14 (0,08, 0,20) 0,11 (0,02, 0,19) 0,21 (0,13, 0,29)	0,080* <0,001 0,011 <0,001
EFW (g) - 28 weeks - 36 weeks	 20,76 (7,04, 24,48) 43,33 (25,20, 61,46)	0,006* 0,003 <0,001

Table 2. Effect of maternal BMI on fetal biometry measures

Fetal biometry

There is a continuous positive linear relationship between maternal BMI and all fetal biometry measures, and estimated fetal weight. A positive relatlionship between maternal BMI and BPD, abdominal area, and EFW velocities was observed (data not shown).

Fetal adiposity

There was no evidence of a strong relationship between maternal BMI and measures of fetal adiposity at 28 weeks' gestation. A significant positive relationship between maternal BMI and measures of fetal adiposity was observed at 36 weeks' gestation.

Outcome	Adjusted estimate (95% CI)	P value
MTTM (cm^2) - 28 weeks - 36 weeks	 0,22 (0,09, 0,35) 0,27 (-0,01, 0,55)	0,740* 0,011 0,060
MTLM (cm^2) - 28 weeks - 36 weeks	 0,17 (0,10, 0,24) 0,06 (-0,08, 0,19)	0,130* <0,001 0,424
MTFM (cm^2) - 28 weeks - 36 weeks	 0,05 (-0,03, 0,14) 0,21 (0,00, 0,41)	0,127* 0,202 0,045
AFM (mm) - 28 weeks - 36 weeks	 0,02 (-0,04, 0,08) 0,17 (0,07, 0,28)	0,006* 0,580 0,001
SSFM (mm) - 28 weeks - 36 weeks	 -0,01 (-0,07, 0,04) 0,26 (0,17, 0,35)	<0,001* 0,630 <0,001

Table 3. Effect of maternal BMI on fetal adiposity measures

Conclusion

There is a continuous, positive linear relationship between increasing maternal BMI and fetal growth measures, with no threshold effects. The effect of increasing maternal BMI on fetal growth measures is seen as early as 20 weeks' gestation, and impacts fetal growth velocity over the third trimester. Maternal BMI is associated with increasing fetal subcutaneous fat measures at 36 weeks', but not 28 weeks' gestation.

References

1. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN et al. Antenatal Lifestyle Advice for Women who are Overweight or Obese: LIMIT Randomised Trial. BMJ. 2014;348:g1285.
2. Dodd JM, Deussen AR, Louises J. A Randomised Trial to Optimise Gestational Weight Gain and Improve Maternal and Infant Health Outcomes through Antenatal Dietary, Lifestyle and Exercise Advice: The OPTIMISE Rnadomised Trial. Nutrients. 2019;11(11).

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Appendix 2

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Evaluating the Mediating Effect of Gestational Diabetes on Fetal Growth and Adiposity in Women who are Overweight or Obese: Findings from the LIMIT Randomised Trial

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Background
Maternal overweight and obesity is associated with an increased risk of adverse outcomes for women and their infants throughout pregnancy, labour and delivery. The fetus and neonate born to women who are overweight or obese are more likely to be born macrosomic or large for gestational age[1, 2]. Gestational diabetes is more common among women who are overweight or obese[3], and infants born to women who develop gestational diabetes are more likely to be born macrosomic or large for gestational age[4]. Little is known, however, about the interplay between maternal overweight and obesity and gestational diabetes on fetal growth and adiposity.

Objective
To describe the mediating effect of maternal gestational diabetes on fetal biometry and adiposity measures among women who are overweight or obese.

Methods
A prospectively conducted randomised trial in which women with a BMI 25kg/m² were randomised to receive a comprehensive dietary and lifestyle intervention or to receive standard care (the LIMIT Randomised trial)[5].
Women presented for an ultrasound assessment at 28 and 36 weeks' gestation at which time standard biometry measures were obtained, in addition to an assessment of fetal body composition. Fetal body composition and adiposity was assessed via mid thigh lean (MTLM) and fat mass (MTFM), abdominal fat mass (AFM) and subscapular fat mass (SSFM). These measurements have been described and validated in previous studies[6,7,8,9]. Gestational diabetes was diagnosed at 28 weeks' gestation following a 75g oral glucose load, if the fasting BSL was >5.5mmol/L or 2 hour BSL was >7.8mmol/L.
These analyses were undertaken in women who were randomised to the standard care / control group, to evaluate mediating effects of maternal GDM on fetal growth and adiposity.
To determine the specific contribution of maternal BMI and GDM on fetal growth and adiposity measures, a mediation analysis was performed. We report the direct effect of maternal BMI and the indirect effect related to a diagnosis of GDM and its subsequent treatment, on measures of fetal biometry and adiposity, and their relative velocities between 28 and 36 weeks' gestation. This relationship is illustrated in Figure 1. BMI was divided into two categories (<30 vs ≥30).

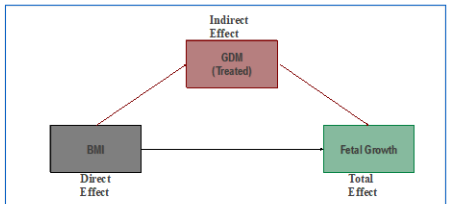

Figure 1. Relationship between maternal BMI and GDM diagnosis on fetal growth and adiposity

Results
Fetal Biometry
There is a direct effect of increasing maternal BMI on increasing fetal HC z-score (p=0.005), with no additional mediating effect from treated gestational diabetes (p=0.183) (Table 1).
Similarly, there is a direct effect from maternal BMI on fetal AC z-score (p=0.001) and estimated fetal weight (p=0.002), with no additional mediating effect from treated gestational diabetes (p=0.532 and 0.901 respectively) (Figure 2; Table 1).

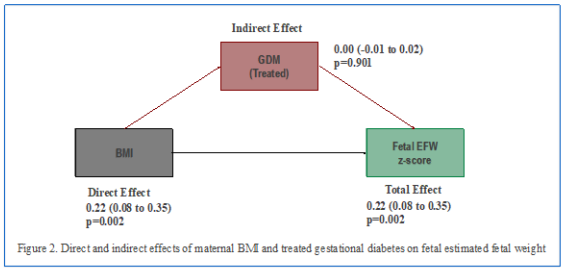

Figure 2. Direct and indirect effects of maternal BMI and treated gestational diabetes on fetal estimated fetal weight

Fetal Biometry Outcome	Adjusted (95% CI)	P value
BPD z-score		
- Total Effect	0.10 (-0.08, 0.28)	0.271
- Direct Effect	0.11 (-0.06, 0.28)	0.198
- Indirect Effect	-0.01 (-0.03, 0.01)	0.315
HC z-score		
- Total Effect	0.17 (0.02, 0.31)	**0.018**
- Direct Effect	0.18 (0.05, 0.31)	**0.005**
- Indirect Effect	-0.01 (-0.02, 0.00)	0.183
AC z-score		
- Total Effect	0.26 (0.11, 0.42)	**0.001**
- Direct Effect	0.26 (0.11, 0.41)	**0.001**
- Indirect Effect	0.01 (-0.01, 0.02)	0.532
FL z-score		
- Total Effect	0.09 (-0.06, 0.24)	0.244
- Direct Effect	0.09 (-0.06, 0.24)	0.256
- Indirect Effect	0.00 (-0.01, 0.02)	0.832
EFW z-score		
- Total Effect	0.22 (0.08, 0.35)	**0.002**
- Direct Effect	0.22 (0.08, 0.35)	**0.002**
- Indirect Effect	0.00 (-0.01, 0.02)	0.901

Table 1. Direct, indirect and total effects of maternal BMI and treated gestational diabetes on fetal biometry outcomes

Fetal Adiposity
In contrast, increasing maternal overweight and obesity was not associated with increased measures of fetal adiposity, with no evidence of an additional mediating effect from treated gestational diabetes (Table 2).

Fetal Adiposity Outcome	Adjusted (95% CI)	P value
MTFM		
- Total Effect	0.07 (-0.50, 0.64)	0.802
- Direct Effect	0.04 (-0.49, 0.58)	0.877
- Indirect Effect	0.03 (-0.03, 0.10)	0.352
AFM		
- Total Effect	0.18 (-0.11, 0.48)	0.223
- Direct Effect	0.17 (-0.11, 0.45)	0.236
- Indirect Effect	0.01 (-0.02, 0.05)	0.402
SSFM		
- Total Effect	0.12 (-0.14, 0.39)	0.362
- Direct Effect	0.13 (-0.12, 0.39)	0.306
- Indirect Effect	-0.01 (-0.04, 0.02)	0.508

Table 2. Direct, indirect and total effects of maternal BMI and treated gestational diabetes on fetal adiposity measures

Conclusions
Increasing maternal BMI exerts an effect on fetal biometry measures however there is no mediating effect attributable to treated GDM on fetal biometry. There is no apparent effect of increasing maternal BMI on fetal adiposity measures, and there is no mediating effect attributable to treated GDM on fetal adiposity.
It is unclear if this represents "saturation" in the risk attributable to the effect of BMI, or if this reflects the effect of treatment of GDM. Either way, this poses a strong case to screen and treat all overweight or obese women for GDM.

References
1. Dodd et al. "Maternal and perinatal health outcomes by body mass index category." Aust N Z J Obstet Gynaecol, 2011.
2. Gaudet et al. "Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis." Biomed Res Int, 2014.
3. Martin, K. E. et al. "The influence of maternal BMI and gestational diabetes on pregnancy outcome." Diabetes Res Clin Pract, 2015.
4. HAPO Study Cooperative Research Group. "Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study: associations with maternal body mass index." BJOG, 2010.
5. Dodd et al. "Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial." BMJ, 2014.
6. Bernstein et al. "Ultrasonographic estimation of fetal body composition for children of diabetic mothers." Invest Radiol, 1991.
7. Hill et al. "Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth." Obstet Gynecol, 1992.
8. Gardeil et al. "Subcutaneous fat in the fetal abdomen as a predictor of growth restriction." Obstet Gynecol, 1999.
9. Higgins et al. "Fetal anterior abdominal wall thickness in diabetic pregnancy." Eur J Obstet Gynecol Reprod Biol, 2008.

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Statement of Authorship

Title of Paper	The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial
Publication Status	Published in the British Journal of Obstetrics and Gynaecology
Publication Details	Poprzeczny AJ, Louise, J, Deussen AR, Dodd JM. The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial. BJOG 2018;125:1558-1566.

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Contribution to the Paper	Assisted in the creation of the concept for the analysis Assisted in the analysis and interpretation of the data Presented the data at an international conference Wrote the manuscript Acted as the corresponding author		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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Contribution to the Paper	Assisted in the design of the study, interpretation of the data and review of the manuscript		
Signature		Date	31/03/2022

BJOG An International Journal of Obstetrics and Gynaecology

DOI: 10.1111/1471-0528.15288
www.bjog.org

Fetal medicine

The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial

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Accepted 29 April 2018. Published Online 14 June 2018.

Objective To describe the mediating effect of maternal gestational diabetes on fetal biometry and adiposity measures among overweight or obese pregnant women.

Design Secondary analysis of the LIMIT randomised trial.

Setting Public hospitals, metropolitan Adelaide, South Australia.

Population Pregnant women with body mass index (BMI) ≥25 kg/m^2 and singleton gestation.

Methods Fetal ultrasound measures at 36 weeks of gestation and baseline BMI from women randomised to the LIMIT trial Standard Care group (n = 912 women) were used to conduct causal mediation analyses using regression-based methods.

Main outcomes measures Ultrasound measures of fetal biometry and adiposity at 36 weeks of gestation.

Results Increased maternal BMI was associated with increased measures of fetal head circumference [direct (unmediated) effect 0.18 (95% CI: 0.05–0.31), P = 0.005; total effect 0.17 (95% CI: 0.02–0.31), P = 0.018], abdominal circumference [direct effect 0.26 (95% CI: 0.11–0.41), P = 0.001; total effect 0.26 (95% CI: 0.11–0.42), P = 0.001] and estimated fetal weight [direct effect 0.22 (95% CI: 0.08–0.35), P = 0.002; total effect 0.22 (95% CI: 0.08–0.35), P = 0.002], with no evidence of mediation by treated gestational diabetes. There was no apparent association between maternal BMI and fetal adiposity measures, or mediation by treated gestational diabetes.

Conclusions We show an important association between increased maternal BMI and fetal growth, not mediated by treated gestational diabetes. There was no association between increased maternal BMI and fetal adiposity measures, or mediation by treated gestational diabetes. Whether these findings represent 'saturation' in the effect of maternal BMI on fetal growth or the effect of treatment of GDM is unclear.

Funding This project was funded by a 4-year project grant from the National Health and Medical Research Council (NHMRC), Australia (ID 519240); The Channel 7 Children's Research Foundation, South Australia; and the US National Institutes of Health (R01 HL094235-01).

Keywords Fetal adiposity, fetal growth, fetal ultrasound, gestational diabetes, maternal obesity.

Tweetable abstract Increased fetal growth associated with maternal obesity is not mediated by gestational diabetes.

Linked article This article is commented on by W Lee, p. 1567 in this issue. To view this mini commentary visit https://doi.org/10.1111/1471-0528.15303.

Please cite this paper as: Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial. BJOG 2018;125:1558–1566.

Introduction

Overweight and obesity, defined as body mass index (BMI) ≥25 and ≥30.0 kg/m^2, respectively, represent a significant public health burden globally.[1] Across developed nations,

Clinical trial registration: Australian and New Zealand Clinical Trials Registry (ACTRN12607000161426).

rates of maternal overweight and obesity are rapidly increasing, with a doubling over the past 20 years.[2,3] In Australia, approximately 50% of women entering pregnancy are overweight or obese.[4,5]

Overweight and obesity are associated with increased risks of adverse maternal and infant outcomes in pregnancy and childbirth.[5] In addition to well-recognised maternal morbidity,[5–7] maternal overweight and obesity are associated with an increased risk of being born large for gestational age or having birthweight over 4 kg,[7,8] perinatal morbidity and mortality,[5,7] and longer term risks of childhood obesity.[9,10]

Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance diagnosed during pregnancy and increases in prevalence with increasing maternal BMI.[7] GDM is associated with adverse perinatal outcomes similar to those seen among women who are overweight or obese in pregnancy, including need for induction of labour and caesarean section, and having an infant born large for gestational age or birthweight over 4 kg.[11–13]

It is unclear whether the combination of maternal overweight or obesity and GDM represents an additive risk in pregnancy, and whether diagnosis and treatment of GDM among women who are overweight or obese modifies this risk. It is also unclear how much of the increased perinatal risk associated with maternal overweight and obesity is attributable to the increased prevalence of GDM. Maternal overweight or obesity and GDM commonly coexist, and have many shared metabolic characteristics. Therefore, it is possible that GDM is on the causal pathway between maternal overweight or obesity and adverse infant outcomes.

We have previously reported that increased maternal BMI is associated with increased fetal growth as measured by ultrasound fetal biometry.[14] The aim of this secondary analysis from the LIMIT randomised trial[15] was to evaluate, among overweight and obese pregnant women, the degree to which the association between maternal BMI and fetal growth and adiposity was mediated via diagnosed and treated GDM.

Methods

Participants

The study protocol and major findings of the LIMIT randomised trial have been published previously.[15–18] Briefly, pregnant women were recruited from three major public metropolitan maternity hospitals in Adelaide, South Australia (Women's and Children's Hospital, Lyell McEwin Hospital and Flinders Medical Centre). Women with a BMI ≥25 kg/m^2 (measured in early pregnancy) and who had a singleton pregnancy between 10^{+0} and 20^{+0} weeks of gestation were eligible for inclusion. Women were excluded if they had a multiple pregnancy, or type 1 or 2 diabetes mellitus diagnosed prior to pregnancy. Written informed consent was obtained from all women, and ethics approval was provided by each hospital review board.

Randomisation

All women presenting for a booking antenatal visit to their participating hospital had their height and weight measured, and BMI calculated. Participating women were randomised to the Lifestyle Advice Group or Standard Care Group using a computer-generated schedule, with stratification for maternal parity, BMI at antenatal booking (25.0–29.9 versus ≥30.0 kg/m^2), and collaborating centre.[16] Briefly, 5474 eligible women were approached to participate, and 2122 women consented and were randomised to the lifestyle advice group (1018 women) or standard care (1104 women).[15] Only data from women randomised to the Standard Care Group were included in the current analyses.

Intervention

Women who were randomised to the Standard Care Group received their pregnancy care according to statewide clinical guidelines, which did not include the routine provision of dietary and lifestyle advice, or information relating to gestational weight gain in pregnancy.[15]

Diagnosis and management of gestational diabetes

Consistent with statewide clinical practice at the time, all women were encouraged to have a fasting 75 g oral glucose tolerance test at 28 weeks of gestation.[19] Using the clinical criteria in place at the time,[19] gestational diabetes was diagnosed if the fasting blood glucose concentration was ≥5.5 mmol/l or the 2-hour blood glucose concentration was ≥7.8 mmol/l. As per clinical practice at the time, women and their care providers were made aware of their results and diagnosis of GDM. Following diagnosis, women were provided with dietary advice and were encouraged to perform home blood glucose monitoring four times daily [before breakfast (fasting) and 2 hours after the start of each meal (postprandial)], with fasting blood glucose measurements targeted between 3.5 and 5.5 mmol/l, and postprandial blood glucose concentrations between 4.0 and 7.0 mmol/l.[19] Medical treatment with insulin or metformin was considered if fasting blood glucose concentrations were ≥5.5 mmol/l once or more per week and/or if postprandial blood glucose concentrations were ≥7.5 mmol/l twice or more per week.[19]

Ultrasound assessment

Women participating in the LIMIT trial attended for a research ultrasound scan at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks of gestation, with fetal biometry and body composition measures obtained as reported

previously.[14] Accurate early assessment of gestational age and estimated date of confinement were calculated based on early pregnancy ultrasound and menstrual period dating. All research ultrasounds were performed by a medical practitioner with specialist or subspecialist training in obstetric ultrasound, while blinded to the participant's allocated treatment group.

Ultrasound assessment included measurements of standard biometry (head circumference, biparietal diameter, abdominal circumference and femur length), measured in accordance with national and international standards of practice.[20] All standard biometry measures were converted to *z*-scores to allow for variation in gestational age and fetal sex, using published Australian population standards.[20,21] Estimated fetal weight was calculated using the Hadlock C formula.[22] Fetal body composition measurements included mid-thigh lean mass (MTLM), mid-thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM), and were obtained by methods described previously.[23–27] In brief, mid-thigh total mass (MTTM), MTLM and MTFM were obtained by taking a longitudinal view of the femur, then rotating the transducer through 90° degrees to obtain a cross-sectional view of the mid-thigh.[24,25] MTFM was measured by taking the total cross-sectional limb area (MTTM) and subtracting MTLM (consisting of the central lean area comprising muscle and bone). Fetal AFM was measured at the level of the abdominal circumference, between fetal mid-axillary lines and anterior to the margins of the ribs.[23,24] This was measured in millimetres and using magnification. The SSFM was obtained by a sagittal view of the fetal trunk, to view the entire longitudinal section of the scapula. The subcutaneous fat tissue measurement was taken at the level of the end of the scapula.[24] We have previously shown good inter-observer variability for these measurements in this cohort.[14]

There was no relevant, published core clinical outcome sets available at the time of planning and performing the LIMIT randomised trial.[15,16]

Statistical analysis

Mediation analyses were performed to investigate the extent to which any associations between maternal BMI and fetal biometry and adiposity measures at 36 weeks of gestation were mediated via diagnosed and treated GDM. For the purposes of analysis, BMI was divided into two categories (25.0–29.9 kg/m^2 and ≥30.0 kg/m^2). When analysing our results using BMI as a continuous variable (data not shown), there was no appreciable difference from the results presented here; therefore, we do not present them here. Regression-based causal mediation models using an extension of the Baron–Kenny method[28] were fitted to determine the effect of BMI category, and any mediating effect of diagnosed and treated GDM, on measures of fetal growth and adiposity, resulting in three estimates for each outcome (Supporting Information Figure S1):

- Total effect, i.e. the total effect of increased BMI on the outcome, including the effect that occurs due to the increased risk of a diagnosis of GDM;
- Direct effect, i.e. the effect of increased BMI on the outcome, without any effect that occurs via the effect of BMI on risk of diagnosis of GDM; and
- Indirect effect, i.e. the effect of GDM on the outcome, independent of any effect of BMI.

In summary, effect estimates represent the difference in mean outcome values at 36 weeks of gestation between BMI categories. Diagnosed and treated GDM was investigated as a potential mediator, that is, a factor that lies along the causal pathway from increased maternal BMI to effect on fetal growth. Maternal BMI was the direct effect, diagnosed and treated GDM was the indirect effect, and the total effect was the combination of the effect seen as a result of increased maternal BMI and GDM.

Analyses were adjusted for centre, parity, SEIFA IRSD Quintile, maternal smoking and maternal age at consent. All analyses were performed using the *paramed* program in STATA v14 (StataCorp, College Station, TX, USA).[28,29]

Participant involvement

Women who participated in the LIMIT randomised trial provided written and verbal feedback relating to their experiences, including attendance for research-based ultrasound examinations, which has informed the design of subsequent studies. Women participating in the trial have also been involved in media events to assist in dissemination of the results to the wider public.

Funding

J. M. Dodd is supported through NHMRC Practitioner Fellowship (ID 627005). This project was funded by a project grant from the National Health and Medical Research Council (NHMRC), Australia (ID 519240); The Channel 7 Children's Research Foundation, South Australia; and the US National Institutes of Health (R01 HL094235-01).

Results

Participant characteristics

These results pertain to the 1104 women randomised to the Standard Care group of the LIMIT study, of whom 912 had at least one research ultrasound performed at 28 and/or 36 weeks of gestation. Twelve women were excluded from mediation analyses as GDM status was missing (11 women) or ultrasound data were missing for all measures used in these analyses (one woman; Figure 1).

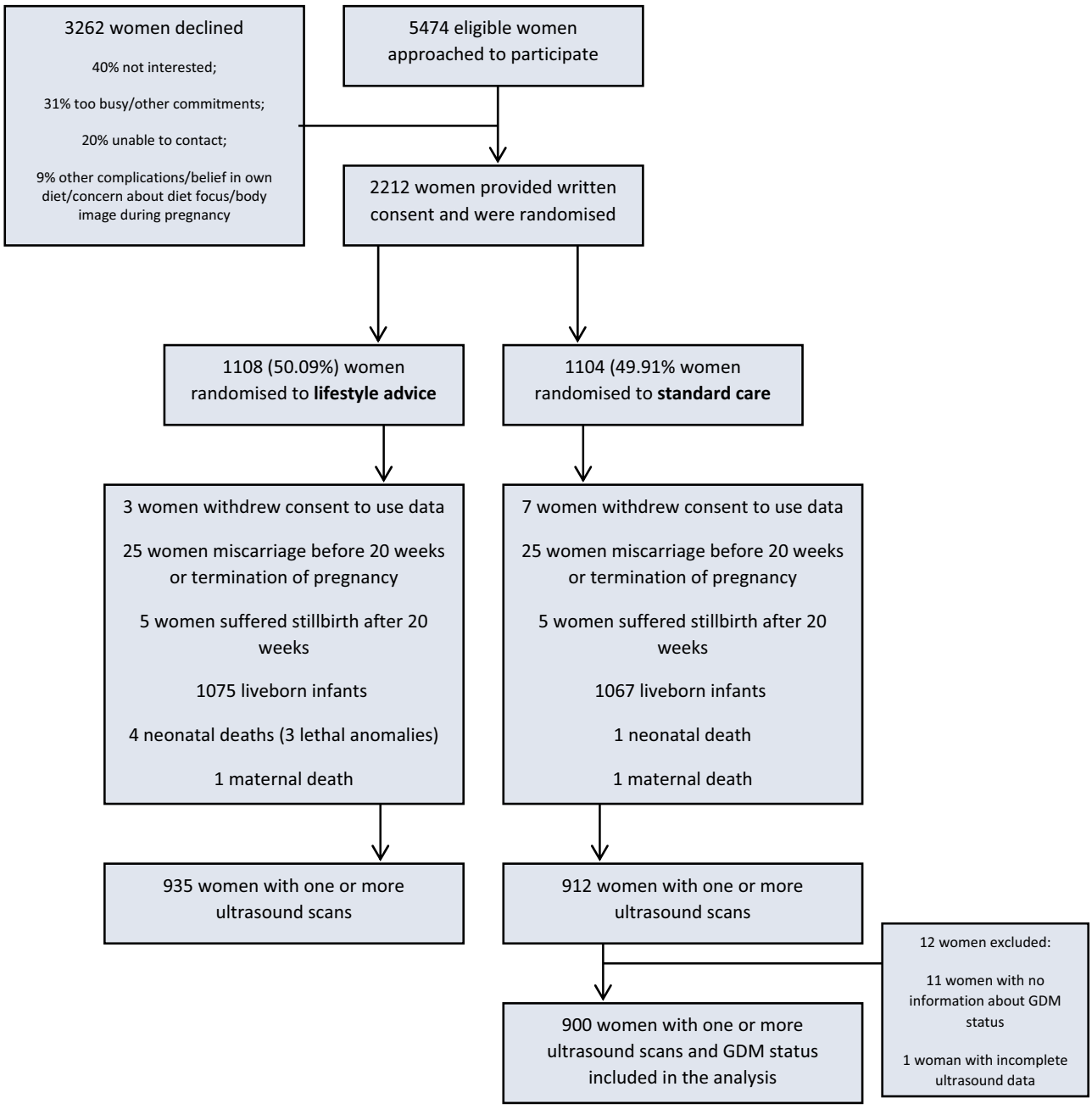

Figure 1. Flow of women eligible for inclusion in the analysis of ultrasound measurements and fetal growth and adiposity.

Baseline characteristics of participants are presented in Table 1. The mean maternal age at trial entry was 29.6 (SD 5.5) years, and mean gestational age at trial entry was 14.6 (SD: 3.0) weeks. The mean BMI of women at trial entry was 32.6 (SD: 6.0) kg/m^2, with a diagnosis of GDM made in 102 women (11.3%). The majority of women were of Caucasian ethnicity [825 women (91.7%)], in their second or subsequent pregnancy [534 women (59.3%)], and were nonsmokers [782 women (86.9%)].

Fetal biometry measures

Maternal BMI was directly associated with fetal head circumference *z*-score, with higher BMI associated with higher head circumference *z*-scores [direct effect 0.18 (95% CI 0.05–0.31), $P = 0.005$; total effect 0.17 (95% CI 0.02–0.31), $P = 0.018$]. However, there was no evidence of an additional mediated effect due to diagnosed and treated GDM [indirect effect −0.01 (95% CI −0.02 to 0.00), $P = 0.183$]. Similar findings were evident for both fetal abdominal

Table 1. Baseline characteristics of Standard Care group participants who attended for one or more research ultrasounds in the LIMIT study

Characteristic	Standard care n = 900	
	n	%
Maternal age at trial entry, years*	29.6	5.5
Gestational age at trial entry, weeks*	14.6	3.0
BMI, kg/m^2*	32.56	6.0
BMI category, kg/m^2**		
25.0–29.9	372	41.3
30.0–34.9	269	29.9
35.0–39.9	152	16.9
≥40.0	107	11.9
Diagnosis of GDM**		
No	798	88.7
Yes	102	11.3
Parity		
0	366	40.7
≥1	534	59.3
Smoking status**		
Yes	99	11.0
No	782	86.9
Unknown	19	2.1
Index of socio-economic disadvantage*,***		
Quintile 1	260	28.9
Quintile 2	220	24.4
Quintile 3	142	15.8
Quintile 4	142	15.8
Quintile 5	136	15.1
Ethnicity**		
Caucasian	825	91.7
Asian	25	2.8
Aboriginal or TSI	10	1.1
Indian/Pakistani/Sri Lankan	27	3.0
African	7	0.8
Other	6	0.7
Public patient**		
Yes	880	97.8
No	20	2.2

BMI, body mass index.
Women were included in the analysis if they had an ultrasound at 28 or 36 weeks of gestation, or both.
*Mean and standard deviation.
**Number and %.
***Socio-economic index as measured by SEIFA: www.abs.gov.au/websitedbs/censushome.nsf/home/seifa2011?opendocument&navpos=260

circumference *z*-scores [direct effect 0.26 (95% CI 0.11–0.41), $P = 0.001$; total effect 0.26 (95% CI 0.11–0.42), $P = 0.001$] and estimated fetal weight *z*-scores [direct effect 0.22 (95% CI 0.08–0.35), $P = 0.002$; total effect 0.22 (95% CI 0.08–0.35), $P = 0.002$], again with no evidence of an additional mediated effect due to diagnosed and treated GDM [abdominal circumference *z*-score indirect effect 0.01 (95% CI −0.01 to 0.02), $P = 0.532$; estimated fetal weight *z*-score indirect effect 0.00 (95% CI −0.01 to 0.02), $P = 0.901$, respectively; Table 2].

Fetal adiposity measures

For women who were overweight or obese, there was no significant association between maternal BMI and fetal adiposity [MTFM direct effect 0.04 cm^2 (95% CI −0.49 to 0.58 cm^2), $P = 0.877$ and total effect 0.07 cm^2 (95% CI −0.50 to 0.64 cm^2), $P = 0.802$; AFM direct effect 0.17 mm (95% CI −0.11 to 0.45 mm), $P = 0.236$ and total effect 0.18 mm (95% CI −0.11 to 0.48 mm), $P = 0.223$; SSFM direct effect 0.13 mm (95% CI −0.12 to 0.39 mm), $P = 0.306$ and total effect 0.12 mm (95% CI −0.14 to 0.39 mm), $P = 0.362$]. There was no evidence of a mediated effect due to diagnosed and treated GDM [MTFM indirect effect 0.03 cm^2 (95% CI −0.03 to 0.10 cm^2), $P = 0.352$; AFM indirect effect 0.01 mm (95% CI −0.02 to 0.05 mm), $P = 0.402$; SSFM indirect effect −0.01 mm (95% CI −0.04 to 0.02 mm), $P = 0.508$; Table 3].

Discussion

Main findings

Our findings demonstrate that in this population of overweight and obese women, although higher maternal BMI

Table 2. Effects of BMI and GDM on ultrasound measures of fetal biometry

Outcome	Adjusted (95% CI)	*P*-value
BPD *z*-score		
Direct effect	0.11 (−0.06 to 0.28)	0.198
Indirect effect	−0.01 (−0.03 to 0.01)	0.315
Total effect	0.10 (−0.08 to 0.28)	0.271
HC *z*-score		
Direct effect	0.18 (0.05–0.31)	0.005
Indirect effect	−0.01 (−0.02 to 0.00)	0.183
Total effect	0.17 (0.02–0.31)	0.018
AC *z*-score		
Direct effect	0.26 (0.11–0.41)	0.001
Indirect effect	0.01 (−0.01 to 0.02)	0.532
Total effect	0.26 (0.11–0.42)	0.001
FL *z*-score		
Direct effect	0.09 (−0.06 to 0.24)	0.256
Indirect effect	0.00 (−0.01 to 0.02)	0.832
Total effect	0.09 (−0.06 to 0.24)	0.244
EFW *z*-score		
Direct effect	0.22 (0.08–0.35)	0.002
Indirect effect	0.00 (−0.01 to 0.02)	0.901
Total effect	0.22 (0.08–0.35)	0.002

AC, abdominal circumference; BMI, body mass index; BPD, biparietal diameter; EFW, estimated fetal weight (Hadlock C formula); FL, femur length; HC, head circumference.
Values are adjusted for centre, parity, SEIFA IRSD Quintile, maternal smoking, and maternal age at consent.

Table 3. Effects of BMI and GDM on ultrasound measures of fetal adiposity

Outcome	Adjusted (95% CI)	*P* value
MTFM		
Direct effect	0.04 (−0.49 to 0.58)	0.877
Indirect effect	0.03 (−0.03 to 0.10)	0.352
Total effect	0.07 (−0.50 to 0.64)	0.802
AFM		
Direct effect	0.17 (−0.11 to 0.45)	0.236
Indirect effect	0.01 (−0.02 to 0.05)	0.402
Total effect	0.18 (−0.11 to 0.48)	0.223
SSFM		
Direct effect	0.13 (−0.12 to 0.39)	0.306
Indirect effect	−0.01 (−0.04 to 0.02)	0.508
Total effect	0.12 (−0.14 to 0.39)	0.362

AFM, abdominal fat mass; BMI, body mass index; MTFM, mid-thigh fat mass; SSFM, subscapular fat mass.
Values are adjusted for centre, parity, SEIFA IRSD Quintile, maternal smoking, and maternal age at consent.

was directly associated with an increase in fetal biometry measures, there was no evidence of an additional mediating effect due to diagnosed and treated GDM. There was no evidence that either maternal BMI category or diagnosed and treated GDM was associated with ultrasound measures of fetal adiposity.

Strengths and limitations

A major strength of our study is the large sample size and prospective data collection. To our knowledge, this is the largest cohort of overweight and obese women who have had serial ultrasound assessment of fetal biometry and adiposity, and the first study to investigate the independent effects of both maternal obesity, and diagnosed and treated GDM on these measurements. We have used a robust research methodology and have obtained standardised ultrasound measures of growth and adiposity, with high levels of inter-observer reliability.[14] Furthermore, diagnosis and management of GDM were standardised according to the local statewide clinical practice in operation during the LIMIT trial.

Our study is not without limitations. This is a secondary analysis of a randomised trial, and validation using other contemporary and appropriately powered cohorts is needed. Although nearly 20% of women are missing some or all of the data required for these analyses, we consider the risk of selection bias to be low, as baseline demographic features of included women are similar to those of the complete randomised cohort.[15] Missing ultrasound data were predominantly due to inability to perform an ultrasound within the specified gestational age window, and unrelated to factors such as maternal BMI, GDM status or fetal abnormalities.

Ultrasound examination in women who are obese is recognised as technically challenging, with increased margins of error at the extremes of fetal growth in particular. Accuracy of fetal anomaly scans has been shown to be reduced,[30,31] and margins of error up to 20% have been reported for estimates of fetal weight in the third trimester.[32] Poor image quality and attenuation of ultrasound by adipose tissue necessitate the use of low-frequency and lower resolution ultrasound settings, often resulting in poorer quality images. Despite this, our measures are robust, with acceptable inter-observer variability in both biometry and adiposity measures in this study population.[14]

Interpretation

Although maternal overweight or obesity and GDM are recognised as risk factors for fetal overgrowth and birthweight ≥4 kg,[7,8,12] their relative contribution and combined effects on fetal growth remain unclear. In a secondary analysis of the HAPO study,[33] both maternal obesity and GDM were independently associated with infant birthweight and adiposity. Notably, the combination of both maternal obesity and GDM resulted in a greater risk than either factor alone, suggesting the effect of maternal BMI is not entirely due to the mediating effect of GDM.[33] This was an observational study, in which clinicians were blinded to maternal glucose tolerance test results, with treatment only offered when blood glucose concentrations were extremely elevated. In contrast, we present data where all women were offered screening, and those with an abnormal glucose tolerance test and diagnosed with GDM, were subsequently offered treatment according to statewide guidelines.

Schaefer-Graf et al.[34] investigated the correlation between maternal BMI, maternal glucose concentrations and fetal and newborn large-for-gestational-age status, in a group of women of all BMI ranges, who were offered treatment following a diagnosis of GDM or impaired glucose tolerance. Although the risk of a large-for-gestational-age infant was significantly higher among women who were obese, this did not appear to be attributable to differences in maternal blood glucose concentrations.[34] Although this was a relatively small retrospective cohort of women, the findings are consistent with our study, providing some evidence to support the clinical importance of treatment of GDM in this high-risk population.

The Pedersen hypothesis of fetal overgrowth[35] suggests that infant macrosomia among diabetic women reflects the effects of fetal hyperglycaemia and subsequent hyperinsulinaemia, resulting from maternal hyperglycaemia. The net effect of increasing birthweight is attributable to the growth-promoting effects of both insulin and glucose. Furthermore, there is strong evidence to indicate that

appropriate treatment and adequate control of blood glucose concentrations in women diagnosed with GDM ameliorate these effects on fetal growth.[36,37]

It is possible that our findings represent 'saturation' in the effect of maternal BMI on fetal biometry and adiposity and that additional effects of treated GDM in an already disordered intrauterine milieu, as observed in women who are overweight or obese, result in no additional effects. Fetuses of women who are overweight or obese are more likely to have abdominal circumferences >90th percentile as early as 20 weeks of gestation, predating the diagnosis and potential effects of GDM.[38] We have previously shown that fetuses of women who are overweight or obese are consistently larger than average, for all ultrasound biometry measures, when using standard population-based growth charts.[14] Maternal overweight and obesity are associated with an early, substantial effect on fetal growth, which is not further attenuated by a diagnosis and treatment of GDM.

There is growing evidence that maternal overweight and obesity influence fetal growth through pathways beyond glucose transport. Obesity is a complex condition in which multiple metabolic pathways are altered, and adipose tissue represents a metabolically active tissue.[39] Metabolic factors including circulating triglycerides,[40,41] leptin[42–44] and adiponectin[45,46] have been associated with higher infant birthweight and adiposity, specifically in women who are overweight or obese, and in women with GDM. The associations with and relative contributions of these factors to fetal fat accumulation, however, are not yet known. Our results may represent an effect of maternal overweight or obesity on fetal growth operating outside of the glucose/insulin pathway.

Conclusion and future work

Our study provides important information on the effects of maternal BMI on fetal biometry, with increased maternal BMI associated with increased fetal growth. However, we did not identify an association between increased maternal BMI and fetal adiposity measures. Furthermore, diagnosed and treated GDM did not appear to confer any additional risk above that associated with maternal BMI alone, and there was no evidence that the effect of maternal BMI on fetal biometry or adiposity was mediated via diagnosed and treated GDM. While this may reflect 'saturation' in the risk attributable to the effects of BMI on fetal growth, it may also represent the effects of a universal policy of screening for and treatment of GDM.

Our work has evaluated the effects of maternal overweight or obesity, and GDM on fetal growth and adiposity, but would be enhanced through comparison of ultrasound parameters of fetal growth among women with a healthy BMI. Future work could also include correlation of ultrasound markers of fetal adiposity with neonatal measures of adiposity in this high-risk population. Other information of interest in determining the pathophysiological mechanisms of fetal overgrowth includes determination of metabolic markers such as triglycerides, leptin, adiponectin and cytokines in women who are overweight or obese, and their associations with fetal and neonatal growth. To this end, it will be possible to interrogate the extensive biobank, which has been established in parallel with the LIMIT trial.[47]

Disclosure of interests

Full disclosure of interests available to view online as supporting information.

Contribution to authorship

Each author has fulfilled the requirements for authorship. JMD and ARD were involved in the study concept and design of the trial, supervision of conduct of the trial and acquisition of data. AJP, JL, ARD, and JMD have been involved equally in the development of the concept of this secondary analysis, analysis and interpretation of data, and critical review of the manuscript, and provide approval of the final submitted version. JL was responsible for conducting the statistical analysis. AJP drafted the manuscript, had full access to all of the study data, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Details of ethics approval

Ethics approval was provided by the Women's and Children's Local Health Network Human Research and Ethics Committee at the Women's and Children's Hospital, REC numbers 1839 (main study) and 2051 (ancillary studies including ultrasound) [1 August 2006 (main study), 11 January 2009 (ancillary studies)], the Central Northern Adelaide Health Service Ethics of Human Research Committee (Lyell McEwin Hospital) REC number 2008033 (15 April 2008), and the Flinders Clinical Research Ethics Committee (Flinders Medical Centre) REC number 128 (8 July 2008).

Funding

This project was funded by a 4-year project grant from the National Health and Medical Research Council (NHMRC), Australia (ID 519240); The Channel 7 Children's Research Foundation, South Australia; and the US National Institutes of Health (R01 HL094235-01). JMD is supported through an NHMRC Practitioner Fellowship (ID 627005). Infrastructure support was provided by The University of Adelaide, and the Women's and Children's Hospital, Flinders Medical Centre, and Lyell McEwin Hospital, Adelaide. The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

Acknowledgements

We are indebted to the 2212 women who participated in the LIMIT randomised trial and acknowledge the following persons who were involved in organising and obtaining the ultrasound scans: R. M. Grivell, A. Newman, L. Kannieappan, R. Earl, C. Staehr, N. Parange, and M. Pinto Barreto.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Relationship between maternal BMI and GDM diagnosis on fetal growth and adiposity. ■

References

1 Wang Y, Beydoun MA, Liang L, Caballero B, Kumanyika SK. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)* 2008;16:2323–30.

2 Heslehurst N, Rankin J, Wilkinson JR, Summerbell CD. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989–2007. *Int J Obes (Lond)* 2010;34:420–8.

3 Kim SY, Dietz PM, England L, Morrow B, Callaghan WM. Trends in pre-pregnancy obesity in nine states, 1993–2003. *Obesity (Silver Spring)* 2007;15:986–93.

4 Scheil WJK, Scott J, Catcheside B, Sage L, Kennare R. Ch II. Mothers and Babies: Characteristics and Outcomes. In: Pregnancy Outcome (Statistics) Unit SH, Government of South Australia(ed.). *Pregnancy Outcome in South Australia 2014*. Adelaide: SA Health; 2016. pp. 28.

5 Callaway LK, Prins JB, Chang AM, McIntyre HD. The prevalence and impact of overweight and obesity in an Australian obstetric population. *Med J Aust* 2006;184:56–9.

6 Cedergren M. Maternal morbid obesity and the risk of adverse pregnancy outcome. *Obstet Gynecol* 2004;103:219–24.

7 Dodd JM, Grivell RM, Nguyen MA, Chan A, Robinson JS. Maternal and perinatal health outcomes by body mass index category. *Aust N Z J Obstet Gynaecol* 2011;51:136–40.

8 Gaudet L, Ferraro ZM, Wen SW, Walker M. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. *Biomed Res Int* 2014;2014:640291.

9 Schellong K, Schulz S, Harder T, Plagemann A. Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. *PLoS One* 2012;7:e47776.

10 Durmus B, Arends LR, Ay L, Hokken-Koelega AC, Raat H, Hofman A, et al. Parental anthropometrics, early growth and the risk of overweight in pre-school children: the Generation R Study. *Pediatr Obes* 2013;8:339–50.

11 Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med* 2008;358:1991–2002.

12 O'Sullivan EP, Avalos G, O'Reilly M, Dennedy MC, Gaffney G, Dunne F. Atlantic diabetes in pregnancy (DIP): the prevalence and outcomes of gestational diabetes mellitus using new diagnostic criteria. *Diabetologia* 2011;54:1670–5.

13 Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. *BMJ* 2016;354:i4694.

14 Grivell RM, Yelland LN, Deussen A, Crowther CA, Dodd JM. Antenatal dietary and lifestyle advice for women who are overweight or obese and the effect on fetal growth and adiposity: the LIMIT randomised trial. *BJOG* 2016;123:233–43.

15 Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. *BMJ* 2014;348:g1285.

16 Dodd JM, Turnbull DA, McPhee AJ, Wittert G, Crowther CA, Robinson JS. Limiting weight gain in overweight and obese women during pregnancy to improve health outcomes: the LIMIT randomised controlled trial. *BMC Pregnancy Childbirth* 2011;11:79.

17 Dodd JM, Cramp C, Sui Z, Yelland LN, Deussen AR, Grivell RM, et al. The effects of antenatal dietary and lifestyle advice for women who are overweight or obese on maternal diet and physical activity: the LIMIT randomised trial. *BMC Med* 2014;12:161.

18 Dodd JM, McPhee AJ, Turnbull D, Yelland LN, Deussen AR, Grivell RM, et al. The effects of antenatal dietary and lifestyle advice for women who are overweight or obese on neonatal health outcomes: the LIMIT randomised trial. *BMC Med* 2014;12:163.

19 SA Health. Gestational Diabetes and Glucose Intolerance in Pregnancy: Diagnosis. In: Health S(ed.). *South Australian Perinatal Practice Guidelines: Diabetes Mellitus and Abnormal Glucose Tolerance*. Adelaide: SA Health, Government of Australia; 2013. pp. 8–9.

20 Australian Society for Ultrasound in Medicine. *Promoting Excellence in Ultrasound-Policy D7: Statement on Normal Ultrasonographic Fetal Measurements*. Sydney; Australian Society for Ultrasound in Medicine: 2007.

21 Hui L. Australian charts for assessing fetal growth: a review. *ASUM Ultrasound Bull* 2008;11:12–8.

22 Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. *Radiology* 1984;150:535–40.

23 Gardeil F, Greene R, Stuart B, Turner MJ. Subcutaneous fat in the fetal abdomen as a predictor of growth restriction. *Obstet Gynecol* 1999;94:209–12.

24 Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, et al. Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies. *Ultrasound Obstet Gynecol* 2003;22:591–7.

25 Bernstein IM, Catalano PM. Ultrasonographic estimation of fetal body composition for children of diabetic mothers. *Invest Radiol* 1991;26:722–6.

26 Higgins MF, Russell NM, Mulcahy CH, Coffey M, Foley ME, McAuliffe FM. Fetal anterior abdominal wall thickness in diabetic pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2008;140:43–7.

27 Hill LM, Guzick D, Boyles D, Merolillo C, Ballone A, Gmiter P. Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth. *Obstet Gynecol* 1992;80:268–71.

28 Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions and implementation with SAS and SPSS macros. *Psychol Methods* 2013;18:137–50.

29 Emsley R, Liu H. PARAMED: Stata module to perform causal mediation analaysis using parametric regression models. Statistical Software Components S457581. 2013. Boston College Department of Economics.

30 Dashe JS, McIntire DD, Twickler DM. Effect of maternal obesity on the ultrasound detection of anomalous fetuses. *Obstet Gynecol* 2009;113:1001–7.

31 Aagaard-Tillery KM, Flint Porter T, Malone FD, Nyberg DA, Collins J, Comstock CH, et al. Influence of maternal BMI on genetic sonography in the FaSTER trial. *Prenat Diagn* 2010;30:14–22.

32 Farrell T, Holmes R, Stone P. The effect of body mass index on three methods of fetal weight estimation. *BJOG* 2002;109:651–7.

33 Catalano PM, McIntyre HD, Cruickshank JK, McCance DR, Dyer AR, Metzger BE, et al. The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. *Diabetes Care* 2012;35:780–6.

34 Schaefer-Graf UM, Heuer R, Kilavuz O, Pandura A, Henrich W, Vetter K. Maternal obesity not maternal glucose values correlates best with high rates of fetal macrosomia in pregnancies complicated by gestational diabetes. *J Perinat Med* 2002;30:313–21.

35 Pedersen J. Weight and length at birth of infants of diabetic mothers. *Acta Endocrinol* 1954;16:330–42.

36 Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. *N Engl J Med* 2009;361:1339–48.

37 Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *N Engl J Med* 2005;352:2477–86.

38 Sovio U, Murphy HR, Smith GC. Accelerated fetal growth prior to diagnosis of gestational diabetes mellitus: a prospective cohort study of nulliparous women. *Diabetes Care* 2016;39:982–7.

39 Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci* 2013;9:191–200.

40 Son GH, Kwon JY, Kim YH, Park YW. Maternal serum triglycerides as predictive factors for large-for-gestational age newborns in women with gestational diabetes mellitus. *Acta Obstet Gynecol Scand* 2010;89:700–4.

41 Vrijkotte TG, Algera SJ, Brouwer IA, van Eijsden M, Twickler MB. Maternal triglyceride levels during early pregnancy are associated with birth weight and postnatal growth. *J Pediatr* 2011;159:736–42.e1.

42 Tsai PJ, Davis J, Bryant-Greenwood G. Systemic and placental leptin and its receptors in pregnancies associated with obesity. *Reprod Sci* 2015;22:189–97.

43 Karakosta P, Chatzi L, Plana E, Margioris A, Castanas E, Kogevinas M. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. *Paediatr Perinat Epidemiol* 2011;25:150–63.

44 Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. *Diabetes Care* 2009;32:1076–80.

45 Ategbo JM, Grissa O, Yessoufou A, Hichami A, Dramane KL, Moutairou K, et al. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. *J Clin Endocrinol Metab* 2006;91:4137–43.

46 Lowe LP, Metzger BE, Lowe WL Jr, Dyer AR, McDade TW, McIntyre HD. Inflammatory mediators and glucose in pregnancy: results from a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. *J Clin Endocrinol Metab* 2010;95:5427–34.

47 Moran LJ, Fraser LM, Sundernathan T, Deussen AR, Louise J, Yelland LN, et al. The effect of an antenatal lifestyle intervention in overweight and obese women on circulating cardiometabolic and inflammatory biomarkers: secondary analyses from the LIMIT randomised trial. *BMC Med* 2017;15:32.

Statement of Authorship

Title of Paper	Fetal growth and adiposity of infants born LGA in three harmonised randomised trials
Publication Status	Submitted for publication in the British Journal of Obstetrics and Gynaecology
Publication Details	Submitted for publication

Principal Author

Name of Principal Author (Candidate)	Dr Amanda Josephine Poprzeczny		
Contribution to the Paper	Assisted in the creation of the concept for the analysis Assisted in the analysis and interpretation of the data Presented the data at an international conference Wrote the manuscript Acted as the corresponding author		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	31/03/2022

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	31/03/2022

Name of Co-Author	Prof Jodie M Dodd		
Contribution to the Paper	Assisted in the design of the study, interpretation of the data and review of the manuscript		
Signature		Date	31/03/2022

TITLE:

Fetal growth and adiposity of infants born LGA in three harmonised randomised trials

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ABSTRACT:

Objective: To compare fetal growth and adiposity measures of infants born LGA with those born not LGA, in order to determine whether the discrepancy at birth was primarily due to a larger size throughout gestation, or instead to different trajectories of fetal growth.

Design: An exploratory analysis of secondary outcomes of fetal growth and adiposity from three harmonized randomized trials – the LIMIT randomized trial, the GRoW randomized trial, and the Optimise randomized trial.

Setting: Three major public metropolitan Adelaide maternity hospitals.

Population: Women were recruited in early pregnancy, with a singleton gestation. Maternal BMI ranged from 18.5-≥40.0 kg/m^2.

Methods: Data were obtained from enrolled women who underwent research ultrasounds at 28 and 36 weeks' gestation. Ultrasound measures of fetal biometry and subcutaneous tissue fat thickness were obtained.

Main outcome measures: Ultrasound measures of fetal biometry and adiposity.

Results: infants born LGA have larger fetal biometry measures, and higher growth trajectories, from 20 weeks' gestation. Similarly, fetal adiposity measures are consistently larger among infants born large for gestational age and these differences increase over time. We did not find evidence that the differences in biometry and adiposity measurements, including changes in magnitude over time, varied according to maternal BMI.

Conclusions: Based on our study results, infants born LGA show differences in fetal biometry and adiposity measures, and growth trajectories, compared to infants born not LGA. These differences are evident from early in pregnancy, suggesting any interventions to prevent LGA likely need to commence earlier in pregnancy, or prior to conception.

Tweetable abstract

Infants born LGA show differences in fetal biometry and adiposity measures, and growth trajectories, compared to infants born not LGA

Keywords:

fetal growth

fetal adiposity

large for gestational age

maternal obesity

Acknowledgements

We are indebted to the 3,260 women who participated in the LIMIT, GRoW and Optimise randomised trial, and attended for research ultrasounds. We acknowledge the work of the following persons who were involved in organising and obtaining the ultrasound scans: R. M. Grivell, A. Newman, L. Kannieappan, R. Earl, C. Staehr, N. Parange, C. O'Brien, E. Raghoudi, H. Waterfall and M.Pinto Barreto.

INTRODUCTION

Large for gestational age (LGA) infants are variably defined as those with a birthweight greater than the 90th, 95th, or 99th percentile for gestational age and sex. Most commonly, LGA infants are defined as those with a birthweight greater than the 90th percentile for gestational age and sex, when compared to a reference population. The incidence of infants born LGA is increasing, with some hypothesizing that this increase is driven by increasing rates of maternal overweight and obesity[98-101, 232]. An infant who is LGA is at an increased risk of shoulder dystocia[103, 104], neonatal hypoglycemia[103, 273], and requiring admission to the nursery[103, 273]. Women who deliver an LGA infant are at an increased risk of requiring delivery by caesarean section[103, 104] and postpartum haemorrhage[103, 273]. Longer term, infants born LGA are at an increased risk of abnormal infant and childhood growth patterns that potentially put them at greater risk of obesity[106, 274-276].

Maternal overweight and obesity, defined as a body mass index (BMI) ≥25 and ≥30 kg/m^2, respectively, represents an independent risk factor for delivering a large for gestational age infant[56, 232, 277, 278]. Across developed nations, rates of maternal overweight and obesity are rapidly increasing, with a doubling over the past 20 years[49, 51]. In Australia, approximately 50% of women entering pregnancy are overweight or obese[57, 279].

An understanding of the fetal growth patterns of infants born LGA may allow for targeting of interventions, in particular timing, to prevent aberrant growth. However, while fetal growth has been studied in infants born LGA to women with diabetes [239-241], there is little published work on fetal growth of infants born LGA to women who do not have diabetes, or among women who are overweight or obese.

This exploratory analysis compared fetal growth and adiposity measures of infants born LGA to those born not LGA, in order to determine whether the discrepancy at birth was primarily due to a larger size throughout gestation, or

instead to different trajectories of growth. Additionally, the potential for these patterns to differ by maternal BMI was explored.

METHODS

The clinical cohort

The analyses reported here use data obtained from research ultrasounds of participants who underwent one or more research ultrasounds in three harmonized randomized controlled trials, the LIMIT[199], GRoW[193] and Optimise[201] randomized trials. Data from women randomized to both standard care and intervention groups were included, from all three trials. The relationship between fetal growth and adiposity and LGA was not expected to differ according to intervention, thus it was considered appropriate to use participants from both groups.

Briefly, women were recruited to one of the three harmonized randomized trials between June 2008 and April 2017 in metropolitan Adelaide, South Australia. Study protocols were purposefully designed so that the studies were sufficiently similar to allow for valid comparisons between them, and for data from the studies to be combined.

Over the time of recruitment of the three randomized trials, local hospital and state guidelines for routine pregnancy care remained consistent[200], with the exception of screening and diagnosis of gestational diabetes[202]. Prior to 2015, the diagnostic criterion for gestational diabetes was a positive 75gm oral glucose tolerance test at 28 weeks' gestation with fasting blood glucose >5.5 mmol/L or 2hr ≥7.8 mmol/L [202]. This change in practice and diagnostic criteria only impacted women recruited to the GrOW and Optimise randomized trials. From 2015, in line with Australian Diabetes in Pregnancy Society (ADIPS) recommendations [203], women were diagnosed with gestational diabetes based on a 75gm oral glucose tolerance test at 28 weeks' gestation with one or more of the following: fasting blood glucose ≥5.1 mmol/L, 1hr ≥10.0 mmol/L, or 2hr ≥8.5 mmol/L[202, 203]. Women diagnosed with gestational diabetes remained in the studies. As per clinical practice at the time, women and their care providers were made aware of their results and diagnosis of GDM. Following diagnosis, women were provided with dietary advice and were encouraged to perform

home blood glucose monitoring four times daily[202]. Advice regarding gestational weight gain was not a part of routine pregnancy care, irrespective of early pregnancy maternal BMI.

In all three trials, women were invited to attend for a research ultrasound at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks of gestation, with fetal biometry and adiposity measures obtained as described below. All research ultrasounds were performed by a medical practitioner with specialist or subspecialist training in obstetric ultrasound, while blinded to the participant's allocated treatment group. A small number of women who underwent a clinical ultrasound during these gestational windows consented to the inclusion of their fetal biometry measurements and did not have a research ultrasound.

Antenatal dietary and lifestyle intervention and control

Women randomized to the Lifestyle Advice group of the LIMIT and Optimise randomized trials[188, 201] received a comprehensive, tailored dietary and lifestyle intervention over the course of their pregnancy, administered by a research dietitian and trained research assistants. The dietary and lifestyle intervention has been described in detail elsewhere[188, 280].

Antenatal metformin as an adjuvant to dietary and lifestyle intervention

All women recruited and randomized to the GRoW randomized trial were overweight or obese in early pregnancy[193] and received the dietary and lifestyle intervention referenced above[199, 280]. In addition, women recruited to the Metformin Group received a supply of oral metformin tablets (500mg) and were instructed to start taking one tablet per day for the first week, then increase to a maximum of two tablets twice daily (maximum 2000mg daily) over four weeks as tolerated, and to continue over the course of the pregnancy. Women allocated to the Placebo Group received a supply of placebo tablets identical in taste and appearance to the metformin tablets, and similarly instructed to start taking one tablet per day for the first week, then increase to a maximum of two tablets twice daily over four weeks as tolerated and continue over the course of the pregnancy. The metformin and placebo tablets were packaged by an

independent pharmaceutical packaging company (Pharmaceutical Packaging Professionals, Victoria).

Fetal ultrasound measures

An accurate gestational age and estimated date of confinement was calculated for each woman based on early pregnancy ultrasound and last menstrual period. Women underwent a routine fetal anomaly scan at 18-20 weeks' gestation, in keeping with South Australian Perinatal Practice Guidelines[200]. Participating women gave permission for fetal biometry results from this scan to be made available to the researchers. All women were invited to attend for a research ultrasound at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks' gestation. A medical practitioner with specialist or subspecialist training in obstetric ultrasound performed all research ultrasounds, and was blinded to the woman's allocated treatment group.

Fetal biometry measures

Fetal biometry measures collected from the routine fetal anomaly scan at 18-20 weeks' gestation included standard measurements of head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). At the research ultrasounds, measurements of standard fetal biometry (HC, BPD, AC, and FL) were obtained in accordance with national and international standards of practice[209]. Biometry measures obtained from the research ultrasounds were converted into z scores to allow for variation in gestational age and fetal sex, using recognised Australian population standards[209, 213, 268]. Estimated fetal weight (EFW) was calculated using the Hadlock C formula[212].

Fetal growth velocities

Fetal growth velocities are presented as the difference between 28 week measures and 36 week measures, calculated as total change / actual number of days between measurements. Velocity z-scores were likewise calculated using recognised Australian population standards where available[281].

Fetal adiposity measures

Fetal subcutaneous tissue fat thickness measurements were obtained at both research ultrasounds. These measurements included mid thigh lean mass (MTLM), mid thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM), and were obtained by methods described previously [118-121, 123, 215, 228]. Mid thigh total, lean and fat mass were obtained by taking a longitudinal view of the femur, then rotating the transducer through 90 degrees to obtain a cross-sectional view of the mid-thigh[119, 120]. MTFM was measured by taking the total cross-sectional limb area (MTTM) and subtracting MTLM (consisting of the central lean area comprising muscle and bone). Fetal AFM was measured at the level of the abdominal circumference, between fetal mid-axillary lines and anterior to the margins of the ribs[119, 121]. This was measured in millimetres and using magnification. The SSFM was obtained by a sagittal view of the fetal trunk, to view the entire longitudinal section of the scapula. The subcutaneous fat tissue measurement was taken at the level of the end of the scapula[119]. We have previously shown good inter-observer variability for these measurements in a similar cohort of women who were overweight or obese in early pregnancy[118].

Definition of LGA

Infants were considered large-for-gestational age (LGA) if their birth weight was >90th percentile for gestational age and infant sex[242].

Statistical analysis

Baseline characteristics of women included in this exploratory analysis were described for the combined cohort. Continuous variables were reported as means and standard deviations, or as medians and interquartile ranges if not normally distributed. Categorical variables were reported as frequencies and percentages.

Detectable effect calculation

The total available sample size for these analyses was all participants from all three studies who had a fetal ultrasound measure for at least one time point. A

total of 3,260 women were included. The overall rate of LGA in this cohort was 17.58%. As a conservative estimate, 2700 participants at a single time point, with the assumed rate of LGA, gives 80% power (with two-sided alpha 0.05) to detect a difference of approximately 0.12 SD in fetal biometry and adiposity measures between LGA and non-LGA infants.

For combined LGA and time effects (i.e. a difference between LGA and non-LGA infants in fetal measures at individual time points, and also a change in this difference over time), data were simulated using a range of effect sizes. The sample size was set at 2700, with two time points, and an LGA rate of 17.58%. Based on 1000 simulations (utilizing a linear regression model with time-by-LGA interaction term and Generalised Estimating Equations to account for correlation due to repeated measures), the power to detect the range of effect sizes was >80% to detect >0.1 SD of difference between LGA and non-LGA, and time-by-LGA interaction effects between 0.5x and 0.75x those of the LGA effect.

Analysis 1: 2-way interaction models

These analyses investigated whether differences in fetal biometry measures between LGA infants and non-LGA infants were evident from 20 weeks' gestation, or whether diverging growth trajectories occur later in pregnancy. Fetal biometry measures at 20, 28, and 36 weeks' gestation; fetal adiposity measures at 28 and 36 weeks' gestation; and fetal growth trajectories were available for analysis. Linear regression models were constructed, with LGA, time and their interaction, with GEEs to account for correlation due to repeated measures. Models were additionally adjusted for maternal BMI, parity, age, intervention group, smoking status, and quintile of socioeconomic disadvantage. A test of interaction between LGA status and time was performed for each growth measure, and the mean difference between LGA and non-LGA infants was estimated separately for each time point. Results are presented as difference in means (LGA – non-LGA), and 95% Confidence Interval.

Analysis 2: 3-way interaction models

Based on the results of the above 2-way interaction analyses, 3-way interaction analyses were performed. This was to investigate whether fetal growth patterns, over time, of infants born LGA, versus infants born non-LGA, varied according to maternal BMI.

These analyses incorporated a three-way interaction between LGA status, time, and maternal BMI. Otherwise, these models were constructed the same as the 2-way interaction models. BMI was modeled as a continuous variable, but three different BMI values (22.0, 27.0 and 35.0 kg/m^2) were used for deriving estimates. There are two sets of interaction p values reported: the first is a test of 3-way interaction. The second set consists of the three tests of LGA-by-time interaction at each level of BMI (that is, at BMI 22.0 kg/m^2, does the difference between LGA and non-LGA vary over time; similarly, at BMI 27.0 kg/m^2, and at BMI 32.0 kg/m^2, does the difference vary over time).

RESULTS

Participant characteristics

We included data from 3,260 women, with baseline characteristics described in table 1. The overall mean BMI at trial entry was 30.71 kg/m^2 (SD 6.92 kg/m^2). The majority of women were in their second or subsequent pregnancy, were non-smokers, and were of Caucasian ethnicity. More than half of women were from two of the most socioeconomically disadvantaged quintiles of the Index of Relatively Socioeconomic Disadvantage[243]. These baseline demographics are generally similar to those of the three randomized trials from which the women were recruited[188, 193, 201]. There were 573 LGA infants (17.58%) born to women in this combined cohort.

Characteristic	Overall Cohort N = 3260
BMI (kg/m^2): Mean (SD)	30.71 (6.92)
BMI Category: N(%)	
18.5-24.9	628 (19.26)
25.0-29.9	1064 (32.64)
30.0-34.9	772 (23.68)
35.0-39.9	478 (14.66)
≥40.0	318 (9.75)
Age at trial entry: Mean (SD)	29.92 (5.41)
Weight at trial entry: Mean (SD)	83.58 (19.96)
Height at trial entry: Mean (SD)	164.86 (6.68)
Multiparous: N(%)	1849 (56.72)
Smoking status: N(%)	
Nonsmoker	2848 (87.36)
Smoker	363 (11.13)
Missing	49 (1.50)
IRSD Quintile: N(%)	
Q1	913 (28.01)
Q2	836 (25.64)
Q3	480 (14.72)
Q4	565 (17.33)
Q5	464 (14.23)
Ethnicity: N(%)	
Caucasian	2770 (84.97)
Non-Caucasian	490 (15.03)
LGA: N(%)	573 (17.58)

Table 1. Baseline characteristics of women from LIMIT, GRoW and Optimise randomized trials who contributed research ultrasound data to the analysis of fetal growth.

BMI, body mass index. SD, standard deviation. N, number. IRSD, Index of Relative Socioeconomic Disadvantage[243]. LGA, large for gestational age.

Fetal biometry measures

Results of the 2-way interaction of LGA and timing of ultrasound analysis are presented in table 2 below. Infants born LGA, in comparison to those born not LGA, were larger in all fetal growth measures, at all time points assessed, and these differences increased over time (table 2). The greatest differences in fetal biometry measures between infants born LGA, and those born not LGA, were seen for fetal AC measures at all time points (table 2). Calculated EFW, which is a function of the fetal biometry measures of BPD, HC, FL, and AC[244], similarly was greater among infants born LGA, compared with those born not LGA, at both 28 and 36 weeks' gestation.

Measure	Not LGA Mean (SD) cm	LGA Mean (SD) cm	Estimate Mean difference (95% CI)	P value
BPD				<0.001*
20 weeks	4.60 (0.33)	4.67 (0.34)	0.06 (0.03, 0.09)	<0.001
28 weeks	7.18 (0.42)	7.35 (0.43)	0.16 (0.12, 0.20)	<0.001
36 weeks	8.87 (0.39)	9.15 (0.35)	0.27 (0.23, 0.30)	<0.001
HC				<0.001*
20 weeks	17.09 (1.13)	17.38 (1.17)	0.25 (0.15, 0.36)	<0.001
28 weeks	26.43 (1.33)	27.02 (1.39)	0.55 (0.41, 0.68)	<0.001
36 weeks	32.02 (1.22)	32.84 (1.09)	0.78 (0.67, 0.89)	<0.001
FL				<0.001*
20 weeks	3.19 (0.29)	3.26 (0.28)	0.06 (0.03, 0.08)	<0.001
28 weeks	5.28 (0.32)	5.40 (0.34)	0.11 (0.08, 0.14)	<0.001
36 weeks	6.85 (0.32)	7.05 (0.30)	0.19 (0.16, 0.22)	<0.001
AC				<0.001*
20 weeks	15.14 (1.23)	15.57 (1.28)	0.35 (0.23, 0.47)	<0.001
28 weeks	24.36 (1.58)	25.49 (1.72)	1.05 (0.88, 1.21)	<0.001
36 weeks	32.28 (1.70)	34.39 (1.91)	2.02 (1.83, 2.20)	<0.001
EFW				<0.001*
28 weeks	1248.15 (215.33)	1390.21 (253.48)	131.99 (107.78, 156.20)	<0.001
36 weeks	2813.16 (355.66)	3258.66 (399.93)	435.44 (396.66, 474.22)	<0.001

Table 2. fetal biometry measures of infants born LGA versus those born not LGA, at 20, 28, and 36 weeks' gestation.

LGA, large for gestational age. CI, confidence interval. SD, standard deviation. BPD, biparietal diameter. HC, head circumference. FL, femur length. AC, abdominal circumference. EFW, estimated fetal weight. * denotes the p value for the interaction term.

Fetal growth z-scores of LGA vs not LGA infants

In keeping with the above, fetal BPD, HC, FL, AC, and EFW z-scores were significantly greater among infants born LGA, compared with those born not LGA, at all time points assessed, and the difference increased over time (data not shown).

With the exception of fetal BPD z-score at 36 weeks' gestation among non-LGA infants [-0.04 (SD 1.15) cm], all z-scores were positive at all time points, indicating that, even among those infants not born LGA, fetuses were larger, on average, than the reference population (data not shown), likely because of the comparatively disproportionate number of women included in this cohort who were overweight or obese. We have previously shown that these women consistently have mean fetal biometry z-scores greater than 0[118].

Fetal adiposity measures of LGA vs not LGA infants

With the exception of AFM measures at 28 weeks' gestation (not LGA mean 3.54 (SD 1.00)mm versus LGA mean 3.67 (SD 0.95)mm; estimated mean difference 0.08 (95% CI -0.05, 0.21)mm; p=0.229], all adiposity measures were statistically significantly greater among infants born LGA, compared with those born not LGA, at all time points, and these differences increased over time (table 3). The magnitude of these estimated mean differences ranged from 0.12 mm (95% CI 0.00, 0.24) for SSFM measurements at 28 weeks' gestation, up to 0.98 mm (95% CI 0.68, 1.27) for MTLM measurements at 28 weeks' gestation (table 3).

Measure	Not LGA Mean (SD) mm	LGA Mean (SD) mm	Estimate Mean difference (95% CI)	P value
MTLM (cm^2)				0.003*
28 weeks	4.82 (1.04)	5.34 (1.07)	0.50 (0.36, 0.64)	<0.001
36 weeks	8.80 (1.93)	9.79 (2.07)	0.98 (0.68, 1.27)	<0.001
MTFM (cm^2)				<0.001*
28 weeks	4.46 (1.19)	5.06 (1.35)	0.58 (0.41, 0.75)	<0.001
36 weeks	10.74 (2.74)	12.76 (3.42)	2.01 (1.53, 2.48)	<0.001
AFM (mm)				<0.001*
28 weeks	3.54 (1.00)	3.67 (0.95)	0.08 (-0.05, 0.21)	0.229
36 weeks	5.46 (1.58)	6.32 (1.72)	0.80 (0.57, 1.04)	<0.001
SSFM (mm)				<0.001*
28 weeks	3.16 (0.85)	3.36 (0.95)	0.12 (0.00, 0.24)	0.047
36 weeks	4.89 (1.38)	5.55 (1.60)	0.59 (0.38, 0.79)	<0.001

Table 3. Fetal adiposity measures in infants born LGA versus those born not LGA.
MTLM, mid-thigh lean mass. MTFM, mid-thigh fat mass. AFM, abdominal fat mass. SSFM, subscapular fat mass. LGA, large for gestational age. SD, standard deviation. CI, confidence interval. * denotes the p value for the interaction term.

Impact of maternal BMI on fetal growth and adiposity measures among infants born LGA, versus those born non-LGA

Fetal biometry measures and fetal biometry measure z-scores

Maternal BMI was not associated with further difference between fetal biometry measures or fetal biometry measures z-scores in infants born LGA, compared with those born not LGA (data not shown)

Fetal adiposity measures

Again, increasing maternal BMI was not associated with further differences seen between fetuses born LGA, compared with those born not LGA, with regards to fetal adiposity measures shown (data not shown)

DISCUSSION

Main findings

Our findings suggest that infants born LGA have larger fetal biometry measures, and higher growth trajectories, evident from 20 weeks' gestation. Similarly, fetal adiposity measures are consistently larger among infants born large for gestational age and these differences increase over time. We did not find evidence that the differences in biometry and adiposity measurements, including changes in magnitude over time, varied according to maternal BMI.

Strengths and limitations

There are many strengths to our work. These results come from a large, prospectively collected cohort of women. While the women whose data were included in this analysis were from multiple trials, they represent a cohort of women whose pregnancies were managed similarly, allowing for such cross-study work to be carried out. Another strength of our work is the inclusion of women across the BMI spectrum, from those with a normal BMI in early pregnancy, to women who were overweight or obese in early pregnancy, allowing consideration of the effect of maternal BMI, across the BMI spectrum, on fetal growth and fetal growth trajectories. While it is known increasing maternal BMI is associated with increasing fetal ultrasound error, we have previously shown good inter-observer correlation between measures obtained in this cohort of women[118]. We acknowledge, however, that this is an exploratory analysis only, and the results should be considered with caution.

Interpretation

These findings add to the growing body of evidence that fetal growth and growth trajectories are "set" from early in pregnancy, and that infants born LGA exhibit differences early. Wong *et al* showed that fetal AC z-scores of infants born LGA were larger on average than those of infants not born LGA, from as early as 18 weeks' gestation[245]. Higher fetal growth rate between the first and second trimester has been associated with an increased risk of infant birth weight greater than 4500gm, or greater than 2 standard deviations above the mean[246].

Taken together, these findings suggest differences in fetal growth may be evident from as early as the first trimester of pregnancy.

Previous work investigating altered fetal growth and fetal growth trajectories among infants born LGA have focused on fetal AC measurements. Madendag *et al* showed that infants born LGA had greater mean AC, and thus EFW, measurements at 26-28 weeks' gestation[132]. Similarly, Caradeux *et al* have shown both fetal AC z-score and AC z-score velocity are predictive of risk of birth of LGA infants[133]. However, the analyses presented here considered all fetal biometry measures, and have shown significant differences in all aspects of fetal growth from as early as 20 weeks' gestation, suggesting fetal skeletal, organ, and adipose tissue growth is impacted by factors contributing to LGA.

Our findings demonstrate that fetal adiposity measures were consistently larger among infants born LGA from early in pregnancy, and that this difference increased with time. These data represent the largest cohort of women who have had longitudinal scans assessing fetal adiposity measures. Interestingly, these results are in agreement with smaller research cohorts. Among a group of 702 Chinese women who had fetal biometry and adiposity measures performed at 28 and 36 weeks' gestation, Chen et al defined population and ethnicity-specific reference ranges for abdominal fat mass and subscapular fat mass measures[247]. The mean AFM measurements among both LGA and not LGA infants in these analyses were significantly larger than the mean AFM measurements in the Chinese population at both 28 and 36 weeks' gestation[247]. The subscapular fat mass measurements among infants born not LGA in these analyses were closer to the population mean subscapular fat mass measurements presented by Chen *et al*, however the mean SSFM measurements among infants born LGA in our population were larger than the population mean's presented by Chen et al at 28 and 36 weeks' gestation[247]. There are some important differences between the population of women undergoing research ultrasounds in our studies and the women recruited to the study by Chen et al[247] that may account for some of these differences. The population providing data here was mainly made up of Caucasian women (n=2770; 84.97%), and mean maternal BMI was 30.71 kg/m^2.

In comparison, the population recruited by Chen et al was made up of solely Asian women, all with a normal BMI (18.5-24.9 kg/m^2)[247].

With regards to fetal subcutaneous tissue thickness measurements, most interest in the literature has focused on abdominal fat mass measurements, and the utility of this measurement in predicting birthweight and risk of LGA infants[121, 248-250]. Abdominal fat mass measures taken during the third trimester have only variably been associated with birthweight and neonatal adiposity measures[127, 129, 251]. This suggests that fetal AFM measurements may not be the most reliable fetal subcutaneous tissue measurement for defining a fetal population at an increased risk of being born LGA, and additional work on other fetal subcutaneous tissue measurements, and overall fetal body composition, are required.

Being born LGA represents an independent risk factor for childhood obesity[252], and may lie on the causal pathway of the intergenerational cycle of obesity[253]. There has been significant interest in the published literature on prevention of LGA birth by antenatal interventions, usually commenced after the first trimester[188, 193, 201, 220, 254]. The findings of this current study, that accelerated growth trajectories of infants born LGA was observed as early as 20 weeks' gestation, provides insight into why antenatal interventions have thus far been ineffective at preventing LGA.

Conclusions and future work

Infants born LGA show increased fetal biometry and adiposity measures, and increased growth trajectories, from early in pregnancy. Antenatal interventions to prevent LGA have potentially started too late to alter fetal growth and growth trajectories. Preconception interventions are an important next step.

DISCLOSURES:

The authors have no conflicts of interest to disclose. The GRoW randomised trial was funded by a National Health and Medical Research Council (NHMRC) project grant (ID 1043181). Jodie Dodd is supported by a NHMRC Practitioner Fellowship (ID 1078980).

Contribution to authorship

Each author fulfills the requirements for authorship. JMD and ARD were involved in the study concept and design of the trials, supervision of conduct of the trial and acquisition of data. AJP developed the concept of this exploratory analysis. AJP, JL, ARD and JMD were involved equally in the analysis and interpretation of data, critical review of the manuscript, and provided approval of the final submitted version. JL was responsible for conducting the statistical analysis. AJP drafted the manuscript, had full access to all of the study data, and takes responsibility for the integrity of the data, and the accuracy of the data analysis.

Clinical trials registrations:

Australian and New Zealand Clinical Trials Registry:
LIMIT – ACTRN12607000161426
GRoW – ACTRN12612001277831
Optimise – ACTRN12614000583640

Details of ethics approval

The study protocols were approved by the Women's and Children's Health Network Human Research and Ethics Committee (LIMIT randomised trial – REC numbers 1839 (main study) and 2051 (ancillary studies including ultrasound); GRoW randomised trial – HREC/12/WCHN/114; Optimise randomised trial – HREC/13/WCHN/152) with local institutional approval at each site. The trials are registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12607000161426, ACTRN12612001277831, ACTRN12614000583640).

Funding

The authors report no conflicts of interest.

J. M. Dodd is supported through NHMRC Practitioner Fellowship (ID 1078980). LIMIT - This project was funded by a project grant from the National Health and Medical Research Council (NHMRC), Australia (ID 519240); The Channel 7 Children's Research Foundation, South Australia; and the US National Institutes of Health (R01 HL094235-01). The GRoW randomised trial was funded by a NHMRC project grant (ID 1043181). The Optimise randomised trial was funded by intramural funding from The University of Adelaide, as well as competitive funding (Lloyd Cox Strategic Research Excellence Award.

REFERENCES

1. Surkan PJ, Hsieh C-C, Johansson ALV, Dickman PW, Cnattingius S. Reasons for Increasing Trends in Large for Gestational Age Births. Obstetrics & Gynecology. 2004;104(4).
2. Ananth CV, Wen SW. Trends in fetal growth among singleton gestations in the United States and Canada, 1985 through 1998. Semin Perinatol. 2002;26(4):260-7.
3. Gaudet L, Ferraro ZM, Wen SW, Walker M. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. BioMed research international. 2014;2014:640291.
4. Ghosh RE, Berild JD, Sterrantino AF, Toledano MB, Hansell AL. Birth weight trends in England and Wales (1986-2012): babies are getting heavier. Arch Dis Child Fetal Neonatal Ed. 2018;103(3):F264-f70.
5. Hadfield RM, Lain SJ, Simpson JM, Ford JB, Raynes-Greenow CH, Morris JM, et al. Are babies getting bigger? An analysis of birthweight trends in New South Wales, 1990–2005. Medical Journal of Australia. 2009;190(6):312-5.
6. Larkin JC, Speer PD, Simhan HN. A customized standard of large size for gestational age to predict intrapartum morbidity. American Journal of Obstetrics and Gynecology. 2011;204(6):499.e1-.e10.
7. Rosen H, Shmueli A, Ashwal E, Hiersch L, Yogev Y, Aviram A. Delivery outcomes of large-for-gestational-age newborns stratified by the presence or absence of gestational diabetes mellitus. International Journal of Gynecology & Obstetrics. 2018;141(1):120-5.
8. WEISSMANN-BRENNER A, SIMCHEN MJ, ZILBERBERG E, KALTER A, WEISZ B, ACHIRON R, et al. Maternal and neonatal outcomes of large for gestational age pregnancies. Acta obstetricia et gynecologica Scandinavica. 2012;91(7):844-9.
9. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005;331(7522):929.
10. Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2005;6(2):143-54.
11. Hediger ML, Overpeck MD, Maurer KR, Kuczmarski RJ, McGlynn A, Davis WW. Growth of infants and young children born small or large for gestational age: findings from the Third National Health and Nutrition Examination Survey. Archives of pediatrics & adolescent medicine. 1998;152(12):1225-31.
12. Moschonis G, Grammatikaki E, Manios Y. Perinatal predictors of overweight at infancy and preschool childhood: the GENESIS study. Int J Obes (Lond). 2008;32(1):39-47.
13. Kim SY, Sharma AJ, Sappenfield W, Wilson HG, Salihu HM. Association of maternal body mass index, excessive weight gain, and gestational diabetes mellitus with large-for-gestational-age births. Obstet Gynecol. 2014;123(4):737-44.
14. Ornoy A. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth

restriction and macrosomia. Reproductive toxicology (Elmsford, NY). 2011;32(2):205-12.
15. Dodd JM, Grivell RM, Nguyen MA, Chan A, Robinson JS. Maternal and perinatal health outcomes by body mass index category. Aust N Z J Obstet Gynaecol. 2011; 51(2):136-40.
16. Heslehurst N, Ells LJ, Simpson H, Batterham A, Wilkinson J, Summerbell CD. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. Bjog. 2007;114(2):187-94.
17. Kim SY, Dietz PM, England L, Morrow B, Callaghan WM. Trends in pre-pregnancy obesity in nine states, 1993-2003. Obesity (Silver Spring). 2007;15(4):986-93.
18. Scheil W JK, Scott J, Catcheside B, Sage L, Kennare R. Pregnancy Outcome in South Australia 2014. In: Pregnancy Outcome (Statistics) Unit SH, Government of South Australia, editor. Adelaide: SA Health; 2016.
19. Callaway LK, Prins JB, Chang AM, McIntyre HD. The prevalence and impact of overweight and obesity in an Australian obstetric population. Med J Aust. 2006;184(2):56-9.
20. Mulder EJH, Koopman CM, Vermunt JK, de Valk HW, Visser GHA. Fetal growth trajectories in Type-1 diabetic pregnancy. Ultrasound in Obstetrics & Gynecology. 2010;36(6):735-42.
21. Hammoud NM, Visser GHA, Peters SAE, Graatsma EM, Pistorius L, de Valk HW. Fetal growth profiles of macrosomic and non-macrosomic infants of women with pregestational or gestational diabetes. Ultrasound in Obstetrics & Gynecology. 2013;41(4):390-7.
22. Lee BH, Park TC, Lee HJ. Association between fetal abdominal circumference and birthweight in maternal hyperglycemia. Acta obstetricia et gynecologica Scandinavica. 2014;93(8):786-93.
23. Dodd JM. Dietary and lifestyle advice for pregnant women who are overweight or obese: the LIMIT randomized trial. Ann Nutr Metab. 2014;64(3-4):197-202.
24. Dodd JM, Louise J, Deussen AR, Grivell RM, Dekker G, McPhee AJ, et al. Effect of metformin in addition to dietary and lifestyle advice for pregnant women who are overweight or obese: the GRoW randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2018.
25. Dodd JM, Deussen AR, Louise J. A Randomised Trial to Optimise Gestational Weight Gain and Improve Maternal and Infant Health Outcomes through Antenatal Dietary, Lifestyle and Exercise Advice: The OPTIMISE Randomised Trial. Nutrients. 2019;11(12).
26. SA Health. South Australian Perinatal Practice Guidelines: Normal Pregnancy, Labour and Puerperium Management. In: Health S, editor. Adelaide, South Australia: SA Health, Government of South Australia; 2015.
27. SA Health. South Australian Perinatal Practice Guidelines: Diabetes Mellitus and Abnormal Glucose Tolerance. In: Health S, editor. Adelaide, South Australia: SA Health, Government of Australia; 2013.
28. Australian Diabetes in Pregnancy Society. ADIPS Consensus Guidelines for the Testing and Diagnosis of Hyperglycaemia in Pregnancy in Australia and New Zealand. 2014 November 2014.

29. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. BMJ. 2014;348:g1285.
30. Dodd JM, Turnbull DA, McPhee AJ, Wittert G, Crowther CA, Robinson JS. Limiting weight gain in overweight and obese women during pregnancy to improve health outcomes: the LIMIT randomised controlled trial. BMC pregnancy and childbirth. 2011;11:79.
31. Australian Society for Ultrasound in Medicine. Promoting Excellence in Ultrasound-Policy D7: Statement on Normal Ultrasonographic Fetal Measurements.: Crows Nest; 2007.
32. Hui L. Australian charts for assessing fetal growth: a review. ASUM Ultrasound Bulletin. 2008 February, 2008:12 - 8.
33. Westerway SC, Davison A, Cowell S. Ultrasonic fetal measurements: new Australian standards for the new millennium. Aust N Z J Obstet Gynaecol. 2000;40(3):297-302.
34. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535-40.
35. Owen P, Harrold AJ, Farrell T. Fetal size and growth velocity in the prediction of intrapartum caesarean section for fetal distress. British journal of obstetrics and gynaecology. 1997;104(4):445-9.
36. Gardeil F, Greene R, Stuart B, Turner MJ. Subcutaneous fat in the fetal abdomen as a predictor of growth restriction. Obstet Gynecol. 1999;94(2):209-12.
37. Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, et al. Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies. Ultrasound Obstet Gynecol. 2003;22(6):591-7.
38. Bernstein IM, Catalano PM. Ultrasonographic estimation of fetal body composition for children of diabetic mothers. Invest Radiol. 1991;26(8):722-6.
39. Higgins MF, Russell NM, Mulcahy CH, Coffey M, Foley ME, McAuliffe FM. Fetal anterior abdominal wall thickness in diabetic pregnancy. Eur J Obstet Gynecol Reprod Biol. 2008;140(1):43-7.
40. Hill LM, Guzick D, Boyles D, Merolillo C, Ballone A, Gmiter P. Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth. Obstet Gynecol. 1992;80(2):268-71.
41. Grivell RM, Yelland LN, Deussen A, Crowther CA, Dodd JM. Antenatal dietary and lifestyle advice for women who are overweight or obese and the effect on fetal growth and adiposity: the LIMIT randomised trial. BJOG. 2016;123(2):233-43. doi: 10.1111/471-0528.13777.
42. Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial. Bjog. 2018.
43. Beeby PJ, Bhutap T, Taylor LK. New South Wales population-based birthweight percentile charts. Journal of paediatrics and child health. 1996;32(6):512-8.
44. Australian Bureau of Statistics. Socio-Economic Indexes for Areas (SEIFA). In: Statistics ABo, editor. Australian Capital Territory: Australian Bureau of Statistics; 2011.

45. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985;151(3):333-7.
46. Wong SF, Chan FY, Oats JJN, McIntyre DH. Fetal Growth Spurt and Pregestational Diabetic Pregnancy. Diabetes Care. 2002;25(10):1681.
47. Pedersen NG, Wøjdemann KR, Scheike T, Tabor A. Fetal growth between the first and second trimesters and the risk of adverse pregnancy outcome. Ultrasound Obstet Gynecol. 2008;32(2):147-54.
48. Madendag Y, Aksoy U, Col Madendag I, Aksoy H. Fetal front-abdominal wall thickness in the second trimester as a predictor of abnormal fetal growth. J Matern Fetal Neonatal Med. 2020:1-7.
49. Caradeux J, Eixarch E, Mazarico E, Basuki TR, Gratacós E, Figueras F. Second- to Third-Trimester Longitudinal Growth Assessment for the Prediction of Largeness for Gestational Age and Macrosomia in an Unselected Population. Fetal diagnosis and therapy. 2018;43(4):284-90.
50. Chen L, Wu J-J, Chen X-H, Cao L, Wu Y, Zhu L-J, et al. Measurement of Fetal Abdominal and Subscapular Subcutaneous Tissue Thickness during Pregnancy to Predict Macrosomia: A Pilot Study. PLOS ONE. 2014;9(3):e93077.
51. Khalifa EA, Hassanein SA, Eid HH. Ultrasound measurement of fetal abdominal subcutaneous tissue thickness as a predictor of large versus small fetuses for gestational age. Egyptian Journal of Radiology and Nuclear Medicine. 2019;50(1):80.
52. Bhat RG, Nathan A, R A, Vasudeva A, Adiga P, Bhat PV, et al. Correlation of fetal abdominal subcutaneous tissue thickness by ultrasound to predict birth weight. J Clin Diagn Res. 2014;8(4):Oc09-11.
53. Köşüş N, Köşüş A, Turhan N. Relation between abdominal subcutaneous fat tissue thickness and inflammatory markers during pregnancy. Arch Med Sci. 2014;10(4):739-45.
54. O'Brien CM, Louise J, Deussen A, Dodd JM. In overweight and obese women, fetal ultrasound biometry accurately predicts newborn measures. Australian and New Zealand Journal of Obstetrics and Gynaecology. 2020;60(1):101-7.
55. O'Connor C, Doolan A, O'Higgins A, Segurado R, Sheridan-Pereiraet M, Turner MJ, et al. Fetal subcutaneous tissue measurements in pregnancy as a predictor of neonatal total body composition. Prenat Diagn. 2014;34(10):952-5.
56. Buhling KJ, Doll I, Siebert G, Catalano PM. Relationship between sonographically estimated fetal subcutaneous adipose tissue measurements and neonatal skinfold measurements. Ultrasound Obstet Gynecol. 2012;39(5):558-62.
57. Sparano S, Ahrens W, De Henauw S, Marild S, Molnar D, Moreno LA, et al. Being macrosomic at birth is an independent predictor of overweight in children: results from the IDEFICS study. Matern Child Health J. 2013;17(8):1373-81.
58. Liu T, Mueller N, Benjamin-Neelon S. Identifying Mediators Underlying the Intergenerational Cycle of Obesity: A Causal Mediation Analysis. Current Developments in Nutrition. 2020;4(Supplement_2):1655-.
59. Poston L, Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, et al. Effect of a behavioural intervention in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. Lancet Diabetes Endocrinol. 2015;3(10):767-77.

60. Walsh JM, McGowan CA, Mahony R, Foley ME, McAuliffe FM. Low glycaemic index diet in pregnancy to prevent macrosomia (ROLO study): randomised control trial. BMJ : British Medical Journal. 2012;345:e5605.

Statement of Authorship

Title of Paper	Effect of metformin in addition to an antenatal diet and lifestyle intervention on fetal growth and adiposity: the GRoW randomised trial
Publication Status	Published in the journal BMC Endocrine Disorders
Publication Details	Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. Effect of metformin in addition to an antenatal diet and lifestyle intervention on fetal growth and adiposity: the GRoW randomised trial. BMC Endocr Disord. 2020 Sep 14;20(1):139.

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Contribution to the Paper	Assisted in the creation of the concept for the analysis Assisted in the analysis and interpretation of the data Presented the data at an international conference Wrote the manuscript Acted as the corresponding author		
Overall percentage (%)	60%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Assisted in the design of the study, interpretation of the data and review of the manuscript		
Signature		Date	31/03/2022

Poprzeczny et al. BMC Endocrine Disorders (2020) 20:139
https://doi.org/10.1186/s12902-020-00618-0

BMC Endocrine Disorders

RESEARCH ARTICLE Open Access

Effect of metformin in addition to an antenatal diet and lifestyle intervention on fetal growth and adiposity: the GRoW randomised trial

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Abstract

Background: The infants born to women who are overweight or obese in pregnancy are at an increased risk of being born macrosomic or large for gestational age. Antenatal dietary and lifestyle interventions have been shown to be ineffective at reducing this risk. Our aim was to examine the effects of metformin in addition to a diet and lifestyle intervention on fetal growth and adiposity among women with a BMI above the healthy range.

Methods: Women who had a body mass index ≥25 kg/m^2 in early pregnancy, and a singleton gestation, were enrolled in the GRoW trial from three public maternity hospitals in metropolitan Adelaide. Women were invited to have a research ultrasounds at 28 and 36 weeks' gestation at which ultrasound measures of fetal biometry and adiposity were obtained. Fetal biometry z-scores and trajectories were calculated. Measurements and calculations were compared between treatment groups. This secondary analysis was pre-specified.

Results: Ultrasound data from 511 women were included in this analysis. The difference in femur length at 36 weeks' gestation was (0.07 cm, 95% CI 0.01–0.14 cm, $p = 0.019$) and this was was statistically significant, however the magnitude of effect was small. Differences between treatment groups for all other fetal biometry measures, z-scores, estimated fetal weight, and adiposity measures at 28 and 36 weeks' gestation were similar.

Conclusions: The addition of metformin to dietary and lifestyle advice in pregnancy for overweight and obese women has no clinically relevant effect on ultrasound measures of fetal biometry or adiposity.

Trial registration: Australian and New Zealand Clinical Trials Registry (ACTRN12612001277831).

Keywords: Fetal growth, Fetal adiposity, Maternal obesity, Metformin, Antenatal interventions

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Background

Women commencing pregnancy overweight or obese, defined as a body mass index (BMI) of ≥ 25 kg/m^2 and ≥ 30 kg/m^2, respectively,have an increased risk of adverse pregnancy and birth outcomes. These adverse outcomes include gestational diabetes (GDM) [1, 2] and hypertensive disorders, including pre-eclampsia [1, 2], during pregnancy and an increased risk of both induction of labour [1, 3] and birth by caesarean section [1, 4]. In developed countries, overweight and obesity effects approximately 50% of women of reproductive age [5–7].

Infants born to women who are overweight or obese are at increased risk of being born macrosomic or large for gestational age (LGA) [1, 8–10], of birth trauma including shoulder dystocia [10, 11], low Apgar scores, requiring resuscitation at birth [1, 8, 10], hypoglycaemia [12, 13] and requiring neonatal intensive care admission [1, 8, 12]. Longer term, maternal overweight and obesity are independent risk factors for childhood obesity [14–16], which may be partially mediated through an increased risk of infant macrosomia [17].

Antenatal dietary and lifestyle interventions have not been effective in reducing the risk of adverse clinical pregnancy and birth outcomes [18–20]. In particular, such interventions have not reduced infant birthweight or risk of an infant born LGA [18].

Oral metformin, a commonly used insulin sensitising agent, has been evaluated as a strategy to improve pregnancy outcomes for overweight and obese women, given similarities in the metabolic environment between both obesity and GDM. However, studies investigating the effects of antenatal metformin have either not reported growth and development in utero [21], or been limited in their analyses [22], resulting in a dearth of information about the fetal effects of antenatal metformin use.

Our group performed a randomised controlled trial investigating the effects of antenatal metformin therapy as an adjuvant to diet and lifestyle advice for women who were overweight or obese in early pregnancy [23]. We have previously reported that adjuvant antenatal metformin therapy is not associated with any differences in birth weight or risk of infants being born large for gestational age [23]. However, whether adjuvant antenatal metformin therapy has an effect on fetal growth, adiposity, and growth velocity, is not known. The aim of this pre-specified analysis of secondary outcomes was to investigate the effect of this combined antenatal intervention on fetal growth and adiposity.

Methods

Participants

The primary findings of the GRoW randomised trial [23] have been published. Women with a singleton, live gestation who were overweight or obese (BMI ≥25.0 kg/m^2) and between 10 and 20 weeks' gestation, were eligible to participate. Women with a multiple pregnancy, pre-existing type 1 or type 2 diabetes mellitus, or a contraindication to taking metformin were excluded from the study.

Randomisation

Potentially eligible women were identified and recruited from three major public maternity hospitals in metropolitan Adelaide – the Women's and Children's Hospital, the Lyell McEwin Hospital, and Flinders Medical Centre. Informed consent was obtained. Randomisation used a central computer-based randomisation service, with variable blocks of four. Women were stratified according to parity (0 vs ≥1), BMI at booking visit (25–29.9 kg/m^2 vs ≥30 kg/m^2), and collaborating centre. Women, their caregivers, and research staff were blinded to treatment allocation.

Intervention

Participating women were randomised to either the metformin group or placebo group. Women recruited to the metformin group received a supply of oral metformin tablets (500 mg) and women allocated to the placebo group received a supply of placebo tablets identical in taste and appearance to the metformin tablets. All women were instructed to start taking one tablet per day increasing to a maximum of two tablets twice daily over four weeks as tolerated, and to continue over the course of the pregnancy [23].

All women received a dietary and lifestyle intervention over the course of the study, which we have described in detail previously [23]. This was an individually tailored intervention, and involved three face-to-face sessions over the course of pregnancy (the initial two with a dietitian, and one with a research assistant at 36 weeks' gestation) and phone calls. Dietary advice was provided, in keeping with Australian dietary standards. This included maintaining a balance of carbohydrates, fat and protein, and reducing energy dense foods high in refined carbohydrates and saturated fats. Women were recommended to consume two servings of fruit, five servings of vegetables, and three servings of dairy each day. Women were encouraged to set and review achievable diet and lifestyle goals and self monitor their progress.

Ultrasound assessment

An accurate gestational age and estimated date of confinement was calculated for each woman based on early pregnancy ultrasound or last menstrual period. Women underwent a routine fetal anomaly scan at 18–20 weeks' gestation, in keeping with South Australian Perinatal Practice Guidelines [24]. Participating women gave permission for fetal biometry results from this scan to be made available to the researchers. All women were

invited to attend for a research ultrasound at 28 (range 26^{+0} to 29^{+6}) and 36 (range 34^{+0} to 37^{+6}) weeks' gestation. A medical practitioner with specialist or subspecialist training in obstetric ultrasound performed all research ultrasounds, and was blinded to the woman's allocated treatment group.

Fetal biometry measures

Fetal biometry measures collected from the routine fetal anomaly scan at 18–20 weeks' gestation included standard measurements of head circumference (HC), biparietal diameter (BPD), abdominal circumference (AC) and femur length (FL). At the research ultrasounds, measurements of standard fetal biometry (HC, BPD, AC, and FL) were obtained in accordance with national and international standards of practice [25]. Biometry measures obtained from the research ultrasounds were converted into z scores to allow for variation in gestational age and fetal sex, using recognised Australian population standards [25, 26]. Estimated fetal weight (EFW) was calculated using the Hadlock C formula [27].

Fetal growth velocities

Fetal growth velocities are presented as the difference between 28 week measures and 36 week measures, calculated as total change / actual number of days between measurements. Velocity z-scores were likewise calculated using recognised Australian population standards where available [28].

Fetal adiposity measures

Fetal subcutaneous tissue fat thickness measurements were obtained at both research ultrasounds. These measurements included mid thigh lean mass (MTLM), mid thigh fat mass (MTFM), abdominal fat mass (AFM), and subscapular fat mass (SSFM), and were obtained by methods described previously [29–34]. Mid thigh total, lean and fat mass were obtained by taking a longitudinal view of the femur, then rotating the transducer through 90 degrees to obtain a cross-sectional view of the mid-thigh [30, 31]. MTFM was measured by taking the total cross-sectional limb area (MTTM) and subtracting MTLM (consisting of the central lean area comprising muscle and bone). Fetal AFM was measured at the level of the abdominal circumference, between fetal mid-axillary lines and anterior to the margins of the ribs [29, 30]. This was measured in millimetres and using magnification. The SSFM was obtained by a sagittal view of the fetal trunk, to view the entire longitudinal section of the scapula. The subcutaneous fat tissue measurement was taken at the level of the end of the scapula [30]. We have previously shown good inter-observer variability for these measurements in a similar cohort of women who were overweight or obese in early pregnancy [33].

Statistical analysis

Baseline characteristics of women included in the analysis were compared descriptively between treatment groups. Continuous variables were reported as means and standard deviations, or as medians and interquartile ranges if not normally distributed. Categorical variables were reported as frequencies and percentages. Analyses were performed on available data on an intention-to-treat basis, with women analysed according to the treatment group into which they were randomised.

Outcomes measured at multiple time points were analysed using linear regression models, with Generalised Estimating Equations to account for correlation due to repeated measures, and a time-by-treatment interaction term to test for differences in treatment effect between time points. Estimates are reported as differences in means (metformin group – placebo group) and 95% confidence intervals for each time point separately, regardless of the significance of the interaction term. Growth velocity outcomes were analysed using linear regression models, with estimates again reported as differences in means (metformin group – placebo group) and 95% confidence intervals. Both unadjusted and adjusted analyses were performed, with adjusted analyses including stratification variables (study centre, BMI category and parity), maternal age at trial entry, smoking status, and Socio-Economic Indexes for Areas Index of Relative Socio-Economic Disadvantage (SEIFA IRSD) quintile as covariates. All analyses were performed using SAS v9.4 (SAS Institute, Cary, NC).

Results

Participant characteristics

A total of 524 women were recruited and randomised in the GRoW randomised trial, with 261 (49.8%) women randomised to the metformin group, and 263 (50.2%) women randomised to the placebo group. Overall, 10 women withdrew, underwent a termination of pregnancy, or suffered a miscarriage before 20 weeks' gestation, resulting in a total of 256 women and their infants in the metformin group and 258 women and their infants in the placebo group who were included in primary analyses (Fig. 1). The present study includes a total of 511 women who attended for one or more research ultrasounds, 255 women from the metformin group and 256 women from the placebo group (Fig. 1).

Baseline demographic characteristics of participating women are shown in Table 1, and were comparable between treatment groups. The median gestational age at trial entry was 16.29 weeks (Interquartile Range (IQR) 14.43–18.00 weeks). The median BMI of the cohort was 32.30 kg/m^2 (IQR 28.90–37.20 kg/m^2), with most women in their second or subsequent pregnancy, non-smokers, and 62.63% from the highest two quintiles of social

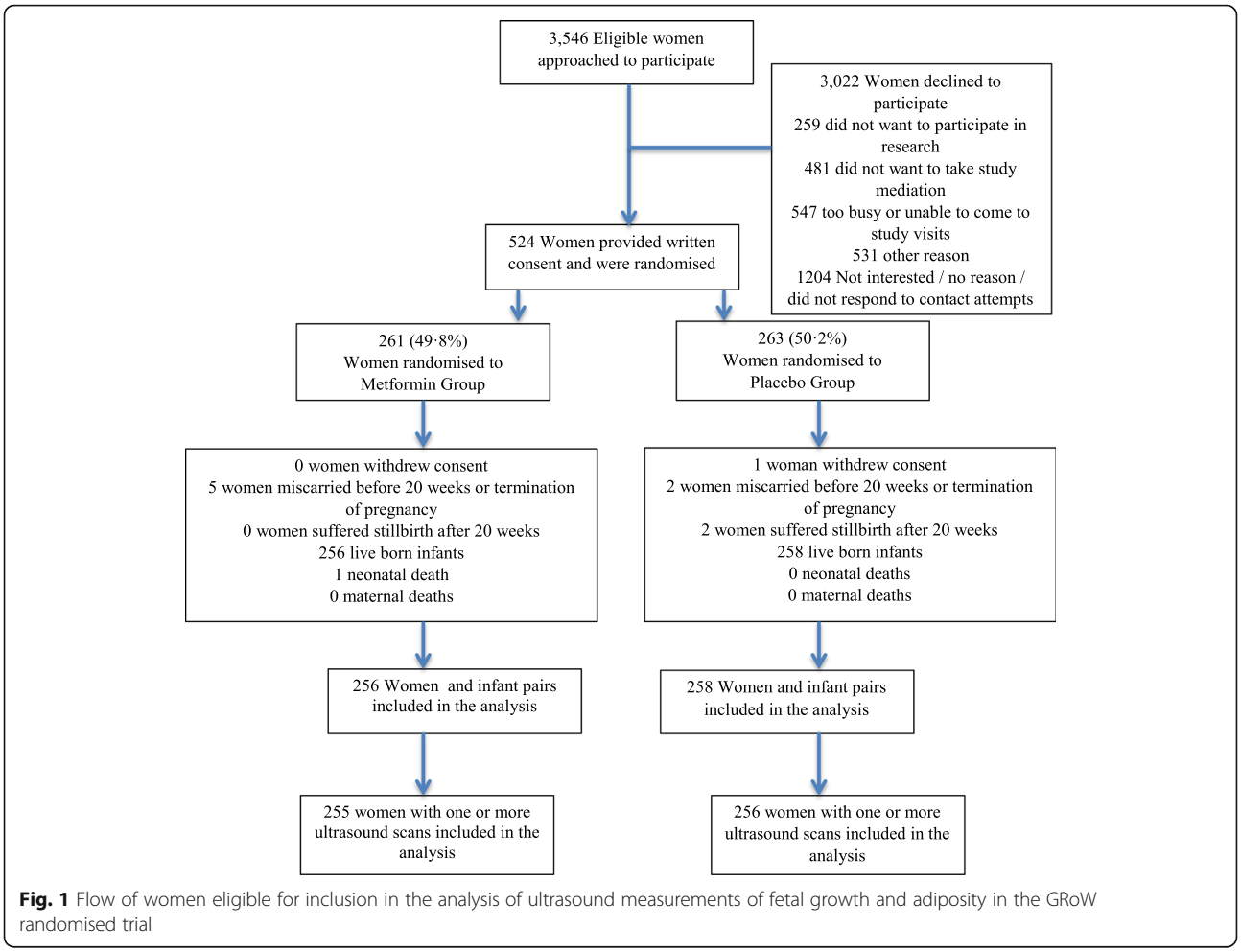

Fig. 1 Flow of women eligible for inclusion in the analysis of ultrasound measurements of fetal growth and adiposity in the GRoW randomised trial

disadvantage [35]. These characteristics are similar to the full randomised cohort [23].

Fetal biometry measures

There were no statistically significant differences in fetal biometry measures of BPD, HC or AC at any of the time points assessed (20 weeks', 28 weeks', and 36 weeks' gestation; Table 2). Measurements of FL were not statistically significantly different at 20 or 28 weeks' gestation (Table 2). Average femur length was statistically significantly greater in the metformin group at 36 weeks' gestation, however the magnitude of the difference was small – only 0.07 (95% CI: 0.01, 0.14) cm. There were no statistically significant differences seen in calculated EFW at 28 or 36 weeks' gestation, by treatment group (Table 2). The estimates of effect size for fetal biometry measures were all less than 0.50 cm and crossed zero (Table 2), suggesting that there were little to no absolute differences in average fetal biometry measures between the metformin and placebo groups, and that the true effect of treatment is not clinically relevant. Similarly, the estimated effect size on estimated fetal weight was only 17.61 (95% CI -57.67, 92.88) gm (Table 2), again suggesting a clinically relevant effect is unlikely.

In keeping with the above findings, there were no statistically significant differences in the fetal biometry z-scores of BPD, HC, FL or AC between the treatment groups, at any of the time points assessed (supplementary Table 1). There were no statistically significant differences in calculated estimated fetal weight z-score at 28 or 36 weeks' gestation, by treatment group (supplementary Table 1). There was no evidence that the treatment effect differed between time points. All fetal biometry measure z-scores were positive in both groups, at all time points, suggesting the fetuses of women in our study were larger on average than the reference population used [26]. With regards to fetal biometry velocities and estimated fetal weight velocity, there were no statistically significant differences by treatment group, either in the raw velocity measurements or velocity for z-score (supplementary Table 2).

Table 1 Baseline characteristics of women recruited to the GRoW randomised trial who attended for one or more research ultrasounds

Characteristic	Metformin Group *N* = 255	Placebo Group *N* = 256	Total *N* = 511
Maternal age (years; mean (SD))	29.88 (5.55)	30.15 (5.38)	30.02 (5.46)
Gestational age at trial entry (weeks) Median (IQR)	16.29 (14.43, 18.00)	16.21 (14.57, 18.07)	16.29 (14.43, 18.00)
BMI (kg/m^2) Median (IQR)	32.40 (28.70, 37.57)	32.05 (29.10, 36.80)	32.30 (28.90, 37.20)
BMI category (kg/m^2) N (%):			
BMI 25.0–29.9	83 (32.55)	83 (32.42)	166 (32.49)
BMI 30.0–34.9	74 (29.02)	83 (32.42)	157 (30.72)
BMI 35.0–39.9	58 (22.75)	48 (18.75)	106 (20.74)
BMI ≥ 40.0	40 (15.69)	42 (16.41)	82 (16.05)
Nulliparity N (%)	88 (34.51)	91 (35.55)	179 (35.03)
Smoker N (%)	24 (9.41)	43 (16.80)	67 (13.11)
Height at trial entry (cm) Mean (SD)	165.24 (6.76)	164.89 (6.78)	165.07 (6.76)
Weight at trial entry (kg) Mean (SD)	92.86 (19.79)	91.87 (19.76)	92.36 (19.76)
Ethnicity N(%)			
Caucasian	209 (81.96)	219 (85.55)	428 (83.76)
Asian	5 (1.96)	7 (2.73)	12 (2.35)
Aboriginal/Torres Strait Islander	6 (2.35)	7 (2.73)	13 (2.54)
African	6 (2.35)	0 (0.00)	6 (1.17)
Other/unknown	29 (11.38)	23 (8.98)	52 (10.18)
SEIFA IRSD Quintile N(%)			
Quintile 1	75 (29.41)	93 (36.33)	168 (32.88)
Quintile 2	78 (30.59)	74 (28.91)	152 (29.75)
Quintile 3	31 (12.16)	30 (11.72)	61 (11.94)
Quintile 4	52 (20.39)	43 (16.80)	95 (18.59)
Quintile 5	19 (7.45)	16 (6.25)	35 (6.85)

Fetal adiposity measures

There was no statistically significant treatment effect on any of the measures of fetal adiposity, at either 28 or 36 weeks' gestation (Table 3). Estimates of effect size for all adiposity measures were close to zero, and the range of the 95% confidence intervals were small, suggesting a clinically meaningful effect is unlikely.

Discussion

Main findings

Our findings demonstrate that, among pregnant women who are overweight or obese, antenatal treatment with oral metformin as an adjunct to dietary and lifestyle advice did not appreciably impact measures of fetal biometry or adiposity, or fetal biometry growth velocities over the third trimester of pregnancy.

Interpretation

Metformin has been used increasingly in the treatment of GDM. The Metformin in Gestational Diabetes (MiG) Trial confirmed the safety and efficacy of metformin use in women with GDM [21]. Oral metformin was not associated with an increased rate of a composite neonatal adverse outcome (made up of neonatal hypoglycaemia, respiratory distress, need for phototherapy, birth trauma, 5-min Apgar < 7, or preterm birth) [21], confirming its safety in pregnancy. Rowan et al also found no significant differences between metformin and insulin with regards to neonatal biometry, circumferences, or skinfold thickness measurements [21].

Subsequent childhood follow up at two years of age, however, revealed selected differences with children exposed to metformin in pregnancy having statistically significantly greater upper arm circumference and subscapular and biceps skinfold thicknesses, and greater fat free mass measurements, in comparison with children exposed to insulin antenatally [36]. It was hypothesised that antenatal metformin caused fat to be stored in subcutaneous sites, resulting in less ectopic or visceral fat [36]. The MiG trial did not report fetal measures of

Table 2 Effect of adjuvant metformin on ultrasound measures of fetal biometry over pregnancy

Outcome	Time point	Metformin group Mean (SD)	Control group Mean (SD)	Unadjusted treatment effect (95% CI)	Unadjusted *P*-value	Adjusted treatment effect (95% CI)	Adjusted P-value
Biparietal diameter (cm)					0.138*		0.102*
	20 weeks	4.66 (0.34)	4.69 (0.35)		0.328		0.232
	28 weeks	7.13 (0.50)	7.15 (0.41)	−0.02 (− 0.10, 0.06)	0.645	− 0.02 (− 0.10, 0.07)	0.671
	36 weeks	8.94 (0.42)	8.88 (0.39)	0.06 (−0.01, 0.14)	0.110	0.07 (− 0.01, 0.14)	0.076
Head circumference (cm)					0.299*		0.200*
	20 weeks	17.38 (1.20)	17.45 (1.36)	−0.07 (−0.29, 0.16)	0.551	−0.09 (− 0.31, 0.13)	0.431
	28 weeks	26.46 (1.55)	26.43 (1.26)	0.04 (−0.22, 0.30)	0.744	0.05 (−0.22, 0.31)	0.727
	36 weeks	32.36 (1.23)	32.20 (1.21)	0.17 (−0.05, 0.40)	0.134	0.19 (−0.04, 0.42)	0.100
Femur length (cm)					0.018*		0.012*
	20 weeks	3.26 (0.30)	3.28 (0.30)	−0.03 (−0.08, 0.02)	0.267	−0.04 (− 0.09, 0.01)	0.127
	28 weeks	5.29 (0.37)	5.29 (0.30)	0.01 (−0.06, 0.07)	0.841	0.00 (−0.06, 0.07)	0.909
	36 weeks	6.92 (0.32)	6.85 (0.33)	0.08 (0.01, 0.14)	0.015	0.07 (0.01, 0.14)	0.019
Abdominal circumference (cm)					0.992*		0.959
	20 weeks	15.56 (1.37)	15.65 (1.29)	−0.09 (−0.32, 0.14)	0.457	−0.10 (− 0.32, 0.12)	0.368
	28 weeks	24.62 (1.75)	24.70 (1.56)	−0.07 (− 0.37, 0.24)	0.669	− 0.05 (− 0.36, 0.26)	0.747
	36 weeks	32.82 (1.87)	32.92 (1.99)	−0.07 (− 0.43, 0.29)	0.690	− 0.07 (− 0.43, 0.29)	0.707
Estimated fetal weight (g)					0.720*		0.756*
	28 weeks	1272.36 (248.33)	1268.81 (201.23)	5.05 (−36.72, 46.82)	0.813	5.40 (−36.93, 47.74)	0.803
	36 weeks	2932.32 (399.97)	2914.27 (417.68)	18.98 (−56.99, 94.95)	0.624	17.61 (−57.67, 92.88)	0.647

*denotes *p* value for test of interaction between treatment and time, i.e. whether treatment effect varies over time

growth and adiposity, however, and our findings refute this hypothesis, as adjuvant antenatal metformin started in the second trimester among women who were overweight or obese in early pregnancy was not associated with any differences in subcutaneous tissue fat measures results in our cohort. Women recruited to the MiG study had an average early pregnancy BMI of 32 kg/m^2 [21], similar to women in the GRoW randomised cohort [23]. In contrast to the MiG study, however, women recruited to the GRoW randomised trial commenced treatment much earlier in pregnancy, with an average gestational age at trial entry of 16 weeks [23].

Our findings are in contrast to those reported in the PregMet2 randomised trial [37], investigating the effect of adjuvant antenatal metformin treatment among women with polycystic ovary syndrome (PCOS), starting in early pregnancy. While the primary outcome of this study was late miscarriage and preterm birth, fetal and neonatal biometry measures were performed in the third trimester and after birth as a secondary outcome [37].

Table 3 Effect of adjuvant metformin treatment on ultrasound fetal adiposity measures over pregnancy

Outcome	Time point	Metformin group Mean (SD)	Control group Mean (SD)	Unadjusted treatment effect (95% CI)	Unadjusted *P*-value	Adjusted treatment effect (95% CI)	Adjusted P-value
Mid-thigh fat mass (cm^2)					0.845*		0.843*
	28 weeks	4.24 (1.03)	4.21 (1.09)	0.04 (−0.27, 0.35)	0.805	0.02 (− 0.29, 0.33)	0.902
	36 weeks	9.87 (2.79)	9.95 (2.47)	−0.05 (− 0.95, 0.84)	0.908	− 0.07 (− 0.94, 0.80)	0.870
Abdominal fat mass (mm)					0.144*		0.199*
	28 weeks	3.60 (1.17)	3.60 (1.08)	0.01 (−0.31, 0.34)	0.935	0.06 (−0.27, 0.39)	0.718
	36 weeks	6.42 (1.41)	5.98 (1.51)	0.42 (−0.05, 0.88)	0.077	0.42 (−0.05, 0.89)	0.079
Subscapular fat mass (mm)					0.919*		0.655*
	28 weeks	3.22 (0.80)	3.20 (0.88)	0.02 (−0.21, 0.25)	0.863	0.03 (−0.19, 0.25)	0.760
	36 weeks	4.84 (1.44)	4.85 (1.48)	−0.00 (− 0.42, 0.41)	0.985	− 0.07 (− 0.48, 0.34)	0.740

*denotes *p* value for test of interaction between treatment and time, i.e. whether treatment effect varies over time

This group found that the fetuses of women exposed to antenatal metformin had statistically significantly larger BPD measurements at 32 weeks' gestation, and greater HC measurements at birth [22]. However, the magnitude of these differences was small – on average, less than one centimetre – and the authors concluded that these differences, while statistically significant, were not likely to be clinically relevant [37]. The population of women recruited to the PregMet2 randomised trial are different to those women recruited to the GRoW randomised trial, in that they all had a diagnosis of PCOS and, while the average BMI at trial entry was 29 kg/m^2, this is lower than the average BMI at trial entry in the GRoW randomised trial, which was 32 kg/m^2 [23]. This study was also smaller than the GRoW randomised trial, recruiting approximately half the number of the GRoW Trial.

Our work builds on that of two other published randomised trials investigating the effect of antenatal metformin among women who were obese in early pregnancy [38, 39]. The Effect of Metformin on Maternal and Fetal Outcomes in Obese Pregnant Women (EMPOWaR) randomised trial [38] randomised women with a BMI ≥ 30 kg/m^2 between 12 and 16 weeks' gestation to receive either metformin up to 2.5 g per day or placebo. They found no significant difference in mean birthweight or birthweight z-score [38], similar to the primary outcome of the GRoW trial [23]. The second trial, Metformin in Obese Nondiabetic Pregnant Women (MOP) trial [39], randomised women with a BMI ≥ 35 kg/m^2 between 12 and 18 weeks' gestation, to metformin up to 3 g daily or placebo. Similarly, they found no statistically significant difference in birthweight z-score or incidence of neonatal LGA [39].

Our group has previously shown, in the LIMIT randomised trial [40], that an antenatal diet and lifestyle intervention in women who were overweight or obese was associated with greater fetal mean mid-thigh fat mass, and a significantly slower rate of subscapular adipose tissue deposition, with no difference in lean thigh mass or abdominal fat mass [33]. All women randomised to the GRoW trial were exposed to the same dietary and lifestyle intervention as was provided in the LIMIT randomised trial [40]. Of interest, results of fetal biometry z scores, estimated fetal weights and subcutaneous fat measures obtained from fetuses of women randomised to the GRoW trial placebo group were similar to those found in fetuses randomised to the diet and lifestyle group of the LIMIT randomised trial [40], providing further support for the robustness of our measurements in these very similar populations. While we have hypothesised previously that an antenatal diet and lifestyle intervention for women who were overweight or obese in early pregnancy may be associated with a more favourable fetal fat phenotype [33], our current findings do not suggest that there is any further effect on fetal adiposity with the addition of metformin to the aforementioned antenatal diet and lifestyle intervention. It is possible that the pathways involved in fetal fat deposition have been "saturated" by the effect of the diet and lifestyle intervention and cannot be further affected by the addition of metformin.

Women recruited to the GRoW randomised trial were, on average, 16 weeks' gestation (Table 1). There is increasing evidence that the preconceptual period is of vital importance to healthy growth and development [41, 42]. Sovio and colleagues [43] demonstrated that the fetuses of women who were obese were more likely to have an abdominal circumference measurement greater than the 90th percentile which was already evident at 20 weeks' gestation. It is possible that interventions need to be commenced prior to conception, to have an appreciable effect on fetal growth and adiposity.

Conclusions

Based on our study results, there is no evidence that the addition of metformin to dietary and lifestyle advice in pregnancy for overweight and obese women has a clinically relevant effect on ultrasound measures of fetal biometry or adiposity.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12902-020-00618-0.

Additional file 1: Supplementary Table 1. Effect of adjuvant antenatal metformin treatment on fetal biometry z-scores across pregnancy. **Supplementary Table 2.** Effect of adjuvant antenatal metformin treatment on fetal biometry velocities across pregnancy.

Abbreviations

GRoW randomised trial: metformin and dietary advice to improve insulin sensitivity and promote gestational restriction of weight among pregnant women who are overweight or obese randomised trial; MoP study: metformin in obese non-diabetes pregnant women randomised trial; MiG study: metformin in gestational diabetes trial; LIMIT randomised trial: limiting weight gain in overweight and obese women during pregnancy to improve health outcomes: a randomised trial; BMI: body mass index; GDM: gestational diabetes; LGA: large for gestational age; HC: head circumference; BPD: biparietal diameter; AC: abdominal circumference; FL: femur length; EFW: estimated fetal weight; MTLM: mid-thigh lean mass; MTFM: mid-thigh fat mass; AFM: abdominal fat mass; SSFM: subscapular fat mass; SEIFA IRSD: socio-economic indexes for areas index of relative socio-economic disadvantage; IQR: interquartile range

Acknowledgements

The following institutions participated in the recruitment of women to the trial:
Women's and Children's Hospital (229 women); The Lyell McEwin Hospital (238 women); Flinders Medical Centre (57 women); and the University of Adelaide.
The following people performed research ultrasounds for the GRoW trial: Grivell R, Poprzeczny A, O'Brien C, Raghoudi E, Waterfall H.
We are indebted to the 524 women who participated in the GRoW randomised trial.

Authors' contributions
Each author fulfils the requirements for authorship. JMD and ARD were involved in the study concept and design of the trial, supervision of conduct of the trial and acquisition of data. AJP, JL, ARD and JMD, have been involved equally in the development of the concept of this secondary analysis, analysis and interpretation of data, critical review of the manuscript, and provide approval of the final submitted version. JL was responsible for conducting the statistical analysis. AJP drafted the manuscript, had full access to all of the study data, and takes responsibility for the integrity of the data, and the accuracy of the data analysis. The author(s) read and approved the final manuscript.

Funding
The GRoW randomised trial was funded by a National Health and Medical Research Council (NHMRC) project grant (ID 1043181). Jodie Dodd is supported by a NHMRC Practitioner Fellowship (ID 1078980). The funding body did not have a role in the design or running of the trial, or the final analysis of results.

Availability of data and materials
The data that supports the findings of this study are available from the authors upon reasonable request and cannot be made publicly available, due to the nature of the data and the ethics approval obtained.

Ethics approval and consent to participate
The Study protocol was approved by the Women's and Children's Health Network Human Research Ethics Committee (HREC/ 12/WCHN/114), with local institutional approval at each site. Written informed consent was obtained from all women who agreed to participate in the trial.

Consent for publication
Not applicable.

Competing interests
The authors have no conflicts of interest to disclose. The GRoW randomised trial was funded by a National Health and Medical Research Council (NHMRC) project grant (ID 1043181). Jodie Dodd is supported by a NHMRC Practitioner Fellowship (ID 1078980).

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Received: 3 December 2019 Accepted: 31 August 2020
Published online: 14 September 2020

References

1. Dodd JM, Grivell RM, Nguyen MA, Chan A, Robinson JS. Maternal and perinatal health outcomes by body mass index category. Aust N Z J Obstet Gynaecol. 2011;51(2):136–40.
2. Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, Mitra A, et al. Obesity and gynaecological and obstetric conditions: umbrella review of the literature. BMJ. 2017;359:j4511.
3. Abenhaim HA, Kinch RA, Morin L, Benjamin A, Usher R. Effect of prepregnancy body mass index categories on obstetrical and neonatal outcomes. Arch Gynecol Obstet. 2007;275(1):39–43.
4. Chu SY, Kim SY, Schmid CH, Dietz PM, Callaghan WM, Lau J, et al. Maternal obesity and risk of cesarean delivery: a meta-analysis. Obesity Rev. 2007;8(5):385–94.
5. Australian Institute of Health and Welfare. A Picture of Overweight and Obesity in Australia 2017. Canberra ACT; 2017. Report No.: PHE 216.
6. Dalenius K, BP, Smith B, Reinold C, Grummer-Strawn L. Pregnancy nutrition surveillance 2010 report. Atlanta: Department of Health and Human Services, Centers for Disease Control and Prevention; 2012.
7. Scheil WJK, Scott J, Catcheside B, Sage L, Kennare R. Pregnancy Outcome in South Australia 2014. In: Government of South Australia, editor. Pregnancy outcome (statistics) unit SH. Adelaide: SA Health; 2016.
8. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. Int J Obes Relat Metab Disord. 2001;25(8):1175–82.
9. Rosenberg TJ, Garbers S, Chavkin W, Chiasson MA. Prepregnancy weight and adverse perinatal outcomes in an ethnically diverse population. Obstet Gynecol. 2003;102(5 Pt 1):1022–7.
10. Cedergren M. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstet Gynecol. 2004;103(2):219–24.
11. Freeman MD, Goodyear SM, Leith WM. A multistate population-based analysis of linked maternal and neonatal discharge records to identify risk factors for neonatal brachial plexus injury. Int J Gynaecol Obstetrics. 2017;136(3):331–6.
12. Callaway LK, Prins JB, Chang AM, McIntyre HD. The prevalence and impact of overweight and obesity in an Australian obstetric population. Med J Aust. 2006;184(2):56–9.
13. Doherty DA, Magann EF, Francis J, Morrison JC, Newnham JP. Pre-pregnancy body mass index and pregnancy outcomes. Int J Gynaecol Obstetrics. 2006;95(3):242–7.
14. Kitsantas P, Gaffney KF. Risk profiles for overweight/obesity among preschoolers. Early Hum Dev. 2010;86(9):563–8.
15. Rooney BL, Mathiason MA, Schauberger CW. Predictors of obesity in childhood, adolescence, and adulthood in a birth cohort. Matern Child Health J. 2011;15(8):1166–75.
16. Jharap VV, Santos S, Steegers EA, Jaddoe VW, Gaillard R. Associations of maternal obesity and excessive weight gain during pregnancy with subcutaneous fat mass in infancy. Early Hum Dev. 2017;108:23–8.
17. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. Pediatrics. 2003;111(3):e221–6.
18. i-WIP Collaborative Group. Effect of diet and physical activity based interventions in pregnancy on gestational weight gain and pregnancy outcomes: meta-analysis of individual participant data from randomised trials. BMJ. 2017;358:j3119.
19. Dodd JM, Cramp C, Sui Z, Yelland LN, Deussen AR, Grivell RM, et al. The effects of antenatal dietary and lifestyle advice for women who are overweight or obese on maternal diet and physical activity: the LIMIT randomised trial. BMC Med. 2014;12(1):161.
20. Poston L, Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, et al. Effect of a behavioural intervention in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. Lancet Diabetes Endocrinol. 2015;3(10):767–77.
21. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008;358(19):2003–15.
22. Hjorth-Hansen A, Salvesen O, Engen Hanem LG, Eggebo T, Salvesen KA, Vanky E, et al. Fetal growth and birth anthropometrics in metformin-exposed offspring born to mothers with PCOS. J Clin Endocrinol Metab. 2018;103(2):740–7.
23. Dodd JM, Louise J, Deussen AR, Grivell RM, Dekker G, McPhee AJ, et al. Effect of metformin in addition to dietary and lifestyle advice for pregnant women who are overweight or obese: the GRoW randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2019;7(1):15–24.
24. Health SA. In: Health S, editor. South Australian perinatal practice guidelines: Normal pregnancy, labour and Puerperium management. Adelaide, South Australia: SA Health, Government of South Australia; 2015.
25. Nisbet D, Robinson H, Halliday J, de CL. Australian Society of Ultrasound in Medicine (ASUM) Policy Statement on normal ultrasonic fetal measurements. Aust N Z J Obstet Gynaecol. 2002;42(1):101–3. https://doi.org/10.1111/j.0004-8666.2002.111_5.x. PMID: 11926635.
26. Hui L. Australian charts for assessing fetal growth: a review. ASUM Ultrasound Bulletin. 2008;2008:12–8.
27. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984;150(2):535–40.
28. Owen P, Harrold AJ, Farrell T. Fetal size and growth velocity in the prediction of intrapartum caesarean section for fetal distress. Br J Obstet Gynaecol. 1997;104(4):445–9.

29. Gardeil F, Greene R, Stuart B, Turner MJ. Subcutaneous fat in the fetal abdomen as a predictor of growth restriction. Obstet Gynecol. 1999;94(2):209–12.
30. Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, et al. Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies. Ultrasound Obstet Gynecol. 2003;22(6):591–7.
31. Bernstein IM, Catalano PM. Ultrasonographic estimation of fetal body composition for children of diabetic mothers. Investig Radiol. 1991;26(8):722–6.
32. Hill LM, Guzick D, Boyles D, Merolillo C, Ballone A, Gmiter P. Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth. Obstet Gynecol. 1992;80(2):268–71.
33. Grivell RM, Yelland LN, Deussen A, Crowther CA, Dodd JM. Antenatal dietary and lifestyle advice for women who are overweight or obese and the effect on fetal growth and adiposity: the LIMIT randomised trial. BJOG. 2016;123(2):233–43. https://doi.org/10.1111/471-0528.13777.
34. Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial. BJOG. 2018;125:1558–66.
35. Australian Bureau of Statistics. Socio-Economic Indexes for Areas (SEIFA). In: Statistics ABo, editor. . Australian Capital Territory: Australian Bureau of Statistics; 2011.
36. Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition at 2 years of age. Diabetes Care. 2011;34(10):2279–84.
37. Lovvik TS, Carlsen SM, Salvesen O, Steffensen B, Bixo M, Gomez-Real F, et al. Use of metformin to treat pregnant women with polycystic ovary syndrome (PregMet2): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2019;7(4):256–66.
38. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2015;3(10):778–86.
39. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus placebo in obese pregnant women without diabetes mellitus. N Engl J Med. 2016;374(5):434–43.
40. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. BMJ. 2014;348:g1285.
41. Stephenson J, Heslehurst N, Hall J, Schoenaker D, Hutchinson J, Cade JE, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet. 2018;391(10132):1830–41.
42. Barker M, Dombrowski SU, Colbourn T, Fall CHD, Kriznik NM, Lawrence WT, et al. Intervention strategies to improve nutrition and health behaviours before conception. Lancet. 2018;391(10132):1853–64.
43. Sovio U, Murphy HR, Smith GC. Accelerated fetal growth prior to diagnosis of gestational diabetes mellitus: a prospective cohort study of nulliparous women. Diabetes Care. 2016;39(6):982–7.

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References

1. Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. Journal of clinical epidemiology. 2011 Apr;64(4):383-94. DOI: 10.1016/j.jclinepi.2010.04.026.
2. World Health Organisation. Fact sheet: Obesity and Overweight. Geneva: World Health Organisation; 2020.
3. Australian Institute of Health and Welfare. Overweight and obesity: an interactive insight. Canberra: AIHW; 2020.
4. Blundell JE, Dulloo AG, Salvador J, Frühbeck G. Beyond BMI--phenotyping the obesities. Obes Facts. 2014;7(5):322-8. DOI: 10.1159/000368783.
5. Goossens GH. The metabolic phenotype in obesity: fat mass, body fat distribution, and adipose tissue function. Obesity facts. 2017;10(3):207-15.
6. Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk--a review of the literature. Eur J Clin Nutr. 2010 Jan;64(1):16-22. DOI: 10.1038/ejcn.2009.68.
7. Piché M-E, Poirier P, Lemieux I, Després J-P. Overview of Epidemiology and Contribution of Obesity and Body Fat Distribution to Cardiovascular Disease: An Update. Progress in Cardiovascular Diseases. 2018 2018/07/01/;61(2):103-13. DOI: https://doi.org/10.1016/j.pcad.2018.06.004.
8. Yusuf S, Hawken S, Ôunpuu S, Bautista L, Franzosi MG, Commerford P, et al. Obesity and the risk of myocardial infarction in 27 000 participants from 52 countries: a case-control study. The Lancet. 2005 2005/11/05/;366(9497):1640-9. DOI: https://doi.org/10.1016/S0140-6736(05)67663-5.
9. Piers LS, Rowley KG, Soares MJ, O'Dea K. Relation of adiposity and body fat distribution to body mass index in Australians of Aboriginal and European ancestry. Eur J Clin Nutr. 2003 Aug;57(8):956-63. DOI: 10.1038/sj.ejcn.1601630.
10. Barlow SE. Expert committee recommendations regarding the prevention, assessment, and treatment of child and adolescent overweight and obesity: summary report. Pediatrics. 2007 Dec;120 Suppl 4:S164-92. DOI: 10.1542/peds.2007-2329C.
11. Australian Institute of Health and Welfare. Impact of overweight and Obesity as a Risk Factor for Chronic Conditions: Australian Burden of Disease Study. In: Welfare AIoHa, editor. Canberra: Australian Institute of Health and Welfare; 2017.
12. Ng M, Fleming T, Robinson M, Thomson B, Gakidou E. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet (London, England). 2014 Aug 30;384(9945):766-81. DOI: 10.1016/s0140-6736(14)60460-8.

13. Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. The Lancet. 2016 2016/04/02/;387(10026):1377-96. DOI: https://doi.org/10.1016/S0140-6736(16)30054-X.
14. World Obesity Federation. Obesity: Missing the 2025 Global Targets. London: World Obesity Federation; 2020 March 2020.
15. World Health Organisation. Obesity and Overweight Fact Sheet. World Health Organisation; 2021 [updated 9 June 2021; cited 2021 28 July 2021]. Available from: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight
16. Australian Bureau of Statistics. National Health Survey 2017-2018. Canberra: Government of Australia; 2018.
17. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. Jama. 2010 Jan 20;303(3):235-41. DOI: 10.1001/jama.2009.2014.
18. Yang L, Colditz GA. Prevalence of Overweight and Obesity in the United States, 2007-2012. JAMA Internal Medicine. 2015;175(8):1412-3. DOI: 10.1001/jamainternmed.2015.2405.
19. Lifestyles Team NHS Digital. Health Survey for England 2019. In: Service GS, editor. Kew, Richmond, Surrey: NHS Digital 2020.
20. WHO. The Double Burden of Malnutrition: Policy Brief. Geneva: World Health Organisation, ; 2017.
21. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. Health affairs (Project Hope). 2009 Sep-Oct;28(5):w822-31. DOI: 10.1377/hlthaff.28.5.w822.
22. Withrow D, Alter DA. The economic burden of obesity worldwide: a systematic review of the direct costs of obesity. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2011 Feb;12(2):131-41. DOI: 10.1111/j.1467-789X.2009.00712.x.
23. Brown WJ, Hockey R, Dobson AJ. Physical activity, Body Mass Index and health care costs in mid-age Australian women. Australian and New Zealand journal of public health. 2008 Apr;32(2):150-5. DOI: 10.1111/j.1753-6405.2008.00192.x.
24. Colagiuri S, Lee CM, Colagiuri R, Magliano D, Shaw JE, Zimmet PZ, et al. The cost of overweight and obesity in Australia. Med J Aust. 2010 Mar 01;192(5):260-4.
25. Bogers RP, Bemelmans WJ, Hoogenveen RT, Boshuizen HC, Woodward M, Knekt P, et al. Association of overweight with increased risk of coronary heart disease partly independent of blood pressure and cholesterol levels: a meta-analysis of 21 cohort studies including more than 300 000 persons. Archives of internal medicine. 2007 Sep 10;167(16):1720-8. DOI: 10.1001/archinte.167.16.1720.
26. Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, et al. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. Lancet (London, England). 2009 Mar 28;373(9669):1083-96. DOI: 10.1016/s0140-6736(09)60318-4.

27. Australian Institute of Health and Welfare. Australian Burden of Disease Study 2015: Interactive data on risk factor burden. Canberra: AIHW; 2020.
28. Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, Murray CJL, et al. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. N Engl J Med. 2017 Jul 06;377(1):13-27. DOI: 10.1056/NEJMoa1614362.
29. Bhaskaran K, Douglas I, Forbes H, dos-Santos-Silva I, Leon DA, Smeeth L. Body-mass index and risk of 22 specific cancers: a population-based cohort study of 5.24 million UK adults. Lancet (London, England). 2014 Aug 30;384(9945):755-65. DOI: 10.1016/s0140-6736(14)60892-8.
30. Lash MM, Armstrong A. Impact of obesity on women's health. Fertil Steril. 2009 May;91(5):1712-6. DOI: 10.1016/j.fertnstert.2008.02.141.
31. Castillo-Martinez L, Lopez-Alvarenga JC, Villa AR, Gonzalez-Barranco J. Menstrual cycle length disorders in 18- to 40-y-old obese women. Nutrition (Burbank, Los Angeles County, Calif). 2003 Apr;19(4):317-20.
32. Yilmaz N, Kilic S, Kanat-Pektas M, Gulerman C, Mollamahmutoglu L. The relationship between obesity and fecundity. J Womens Health (Larchmt). 2009 May;18(5):633-6. DOI: 10.1089/jwh.2008.1057.
33. Wise LA, Rothman KJ, Mikkelsen EM, Sørensen HT, Riis A, Hatch EE. An internet-based prospective study of body size and time-to-pregnancy. Hum Reprod. 2010 Jan;25(1):253-64. DOI: 10.1093/humrep/dep360.
34. Gesink Law DC, Maclehose RF, Longnecker MP. Obesity and time to pregnancy. Hum Reprod. 2007 Feb;22(2):414-20. DOI: 10.1093/humrep/del400.
35. Lintsen AM, Pasker-de Jong PC, de Boer EJ, Burger CW, Jansen CA, Braat DD, et al. Effects of subfertility cause, smoking and body weight on the success rate of IVF. Hum Reprod. 2005 Jul;20(7):1867-75. DOI: 10.1093/humrep/deh898.
36. Bellver J, Ayllón Y, Ferrando M, Melo M, Goyri E, Pellicer A, et al. Female obesity impairs in vitro fertilization outcome without affecting embryo quality. Fertil Steril. 2010 Feb;93(2):447-54. DOI: 10.1016/j.fertnstert.2008.12.032.
37. Alvarez-Blasco F, Botella-Carretero JI, San Millan JL, Escobar-Morreale HF. Prevalence and characteristics of the polycystic ovary syndrome in overweight and obese women. Archives of internal medicine. 2006 Oct 23;166(19):2081-6. DOI: 10.1001/archinte.166.19.2081.
38. Jean Hailes Foundation for Women's Health. Evidence-based guidelines for the assessment and management of polycystic ovary syndrome. Melbourne: PCOS Australian Alliance; 2011.
39. Aune D, Navarro Rosenblatt DA, Chan DS, Vingeliene S, Abar L, Vieira AR, et al. Anthropometric factors and endometrial cancer risk: a systematic review and dose-response meta-analysis of prospective studies. Annals of oncology : official journal of the European Society for Medical Oncology. 2015 Aug;26(8):1635-48. DOI: 10.1093/annonc/mdv142.
40. Lacey JV, Jr., Swanson CA, Brinton LA, Altekruse SF, Barnes WA, Gravitt PE, et al. Obesity as a potential risk factor for adenocarcinomas and squamous cell carcinomas of the uterine cervix. Cancer. 2003 Aug 15;98(4):814-21. DOI: 10.1002/cncr.11567.

41. Poorolajal J, Jenabi E. The association between BMI and cervical cancer risk: a meta-analysis. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP). 2016 May;25(3):232-8. DOI: 10.1097/cej.0000000000000164.
42. Kalliala I, Markozannes G, Gunter MJ, Paraskevaidis E, Gabra H, Mitra A, et al. Obesity and gynaecological and obstetric conditions: umbrella review of the literature. Bmj. 2017 Oct 26;359:j4511. DOI: 10.1136/bmj.j4511.
43. RANZCOG. Management of Obesity in Pregnancy. C-Obs 49 ed: Royal Australian and New Zealand College of Obstetricians and Gynaecologists; 2017.
44. Fattah C, Farah N, Barry SC, O'Connor N, Stuart B, Turner MJ. Maternal weight and body composition in the first trimester of pregnancy. Acta obstetricia et gynecologica Scandinavica. 2010 Jul;89(7):952-5. DOI: 10.3109/00016341003801706.
45. Denison F, Aedla N, Keag O, Hor K, Reynolds R, Milne A, et al. Care of Women with Obesity in Pregnancy. Green-top Guideline No. 72. . BJOG. 2018.
46. Han E, Abrams B, Sridhar S, Xu F, Hedderson M. Validity of Self-Reported Pre-Pregnancy Weight and Body Mass Index Classification in an Integrated Health Care Delivery System. Paediatr Perinat Epidemiol. 2016 Jul;30(4):314-9. DOI: 10.1111/ppe.12286.
47. Schieve LA, Perry GS, Cogswell ME, Scanion KS, Rosenberg D, Carmichael S, et al. Validity of self-reported pregnancy delivery weight: an analysis of the 1988 National Maternal and Infant Health Survey. NMIHS Collaborative Working Group. Am J Epidemiol. 1999 Nov 01;150(9):947-56.
48. Mandujano A, Huston-Presley L, Waters TP, Catalano PM. Women's reported weight: is there a discrepancy? J Matern Fetal Neonatal Med. 2012 Aug;25(8):1395-8. DOI: 10.3109/14767058.2011.636099.
49. Heslehurst N, Ells LJ, Simpson H, Batterham A, Wilkinson J, Summerbell CD. Trends in maternal obesity incidence rates, demographic predictors, and health inequalities in 36,821 women over a 15-year period. Bjog. 2007 Feb;114(2):187-94. DOI: 10.1111/j.1471-0528.2006.01199.x.
50. NHS Maternity Statistics. NHS Maternity Statistics, England 2018-2019. In: NHS Digitial, editor.
51. Kim SY, Dietz PM, England L, Morrow B, Callaghan WM. Trends in pre-pregnancy obesity in nine states, 1993-2003. Obesity (Silver Spring, Md). 2007 Apr;15(4):986-93. DOI: 10.1038/oby.2007.621.
52. Driscoll AK, Gregory ECW. Increases in Prepregnancy Obesity: United States, 2016-2019. In: U.S. Centers for Disease Control and Prevention, editor.2020.
53. Australian Institute of Health and Welfare. Australia's mothers and babies. Canberra: AIHW; 2021.
54. Pregnancy Outcome Unit. Pregnancy Outcome in South Australia. In: Pregnancy Outcome (Statistics) Unit SH, Government of South Australia, editor. Adelaide: Wellbeing SA, Government of South Australia; 2019.
55. McIntyre HD, Gibbons KS, Flenady VJ, Callaway LK. Overweight and obesity in Australian mothers: epidemic or endemic? Med J Aust. 2012 Feb 20;196(3):184-8.

56. Dodd JM, Grivell RM, Nguyen MA, Chan A, Robinson JS. Maternal and perinatal health outcomes by body mass index category. Aust N Z J Obstet Gynaecol. 2011; 51(2):136-40.
57. Callaway LK, Prins JB, Chang AM, McIntyre HD. The prevalence and impact of overweight and obesity in an Australian obstetric population. The Medical journal of Australia. 2006 Jan 16;184(2):56-9.
58. Abenhaim HA, Kinch RA, Morin L, Benjamin A, Usher R. Effect of prepregnancy body mass index categories on obstetrical and neonatal outcomes. Archives of gynecology and obstetrics. 2007 Jan;275(1):39-43. DOI: 10.1007/s00404-006-0219-y.
59. Metwally M, Ong KJ, Ledger WL, Li TC. Does high body mass index increase the risk of miscarriage after spontaneous and assisted conception? A meta-analysis of the evidence. Fertil Steril. 2008 Sep;90(3):714-26. DOI: 10.1016/j.fertnstert.2007.07.1290.
60. Chen CN, Chen HS, Hsu HC. Maternal Prepregnancy Body Mass Index, Gestational Weight Gain, and Risk of Adverse Perinatal Outcomes in Taiwan: A Population-Based Birth Cohort Study. Int J Environ Res Public Health. 2020 Feb 14;17(4). DOI: 10.3390/ijerph17041221.
61. Denison FC, Norwood P, Bhattacharya S, Duffy A, Mahmood T, Morris C, et al. Association between maternal body mass index during pregnancy, short-term morbidity, and increased health service costs: a population-based study. Bjog. 2014 Jan;121(1):72-81; discussion 2. DOI: 10.1111/1471-0528.12443.
62. Melchor I, Burgos J, del Campo A, Aiartzaguena A, Gutiérrez J, Melchor JC. Effect of maternal obesity on pregnancy outcomes in women delivering singleton babies: a historical cohort study. Journal of Perinatal Medicine. 2019;47(6):625-30. DOI: doi:10.1515/jpm-2019-0103.
63. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstetrics and gynecology. 2004 Feb;103(2):219-24. DOI: 10.1097/01.aog.0000107291.46159.00.
64. Chu SY, Kim SY, Schmid CH, Dietz PM, Callaghan WM, Lau J, et al. Maternal obesity and risk of cesarean delivery: a meta-analysis. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2007 Sep;8(5):385-94. DOI: 10.1111/j.1467-789X.2007.00397.x.
65. Blondon M, Harrington LB, Boehlen F, Robert-Ebadi H, Righini M, Smith NL. Pre-pregnancy BMI, delivery BMI, gestational weight gain and the risk of postpartum venous thrombosis. Thromb Res. 2016 Sep;145:151-6. DOI: 10.1016/j.thromres.2016.06.026.
66. Catalano PM, Hauguel-De Mouzon S. Is it time to revisit the Pedersen hypothesis in the face of the obesity epidemic? Am J Obstet Gynecol. 2011 Jun;204(6):479-87. DOI: 10.1016/j.ajog.2010.11.039.
67. Ryan EA. Hormones and insulin resistance during pregnancy. Lancet (London, England). 2003;362(9398):1777-8.
68. Lowe LP, Metzger BE, Lowe WL, Jr., Dyer AR, McDade TW, McIntyre HD. Inflammatory mediators and glucose in pregnancy: results from a subset of the Hyperglycemia and Adverse Pregnancy Outcome (HAPO) Study. The Journal of clinical endocrinology and metabolism. 2010 Dec;95(12):5427-34. DOI: 10.1210/jc.2010-1662.

69. Martin KE, Grivell RM, Yelland LN, Dodd JM. The influence of maternal BMI and gestational diabetes on pregnancy outcome. Diabetes research and clinical practice. 2015 Jun;108(3):508-13. DOI: 10.1016/j.diabres.2014.12.015.
70. Lopez-Jaramillo P, Barajas J, Rueda-Quijano SM, Lopez-Lopez C, Felix C. Obesity and Preeclampsia: Common Pathophysiological Mechanisms. Frontiers in physiology. 2018;9:1838-. DOI: 10.3389/fphys.2018.01838. Cited in: PubMed.
71. Chu SY, Kim SY, Lau J, Schmid CH, Dietz PM, Callaghan WM, et al. Maternal obesity and risk of stillbirth: a metaanalysis. Am J Obstet Gynecol. 2007 Sep;197(3):223-8. DOI: 10.1016/j.ajog.2007.03.027.
72. Flenady V, Koopmans L, Middleton P, Froen JF, Smith GC, Gibbons K, et al. Major risk factors for stillbirth in high-income countries: a systematic review and meta-analysis. Lancet (London, England). 2011 Apr 16;377(9774):1331-40. DOI: 10.1016/s0140-6736(10)62233-7.
73. Nohr EA, Bech BH, Davies MJ, Frydenberg M, Henriksen TB, Olsen J. Prepregnancy obesity and fetal death: a study within the Danish National Birth Cohort. Obstet Gynecol. 2005 Aug;106(2):250-9. DOI: 10.1097/01.aog.0000172422.81496.57.
74. Sebire NJ, Jolly M, Harris JP, Wadsworth J, Joffe M, Beard RW, et al. Maternal obesity and pregnancy outcome: a study of 287,213 pregnancies in London. International journal of obesity and related metabolic disorders : journal of the International Association for the Study of Obesity. 2001 Aug;25(8):1175-82. DOI: 10.1038/sj.ijo.0801670.
75. Hibbard JU, Gilbert S, Landon MB, Hauth JC, Leveno KJ, Spong CY, et al. Trial of labor or repeat cesarean delivery in women with morbid obesity and previous cesarean delivery. Obstet Gynecol. 2006 Jul;108(1):125-33. DOI: 10.1097/01.aog.0000223871.69852.31.
76. Jensen DM, Damm P, Sorensen B, Molsted-Pedersen L, Westergaard JG, Ovesen P, et al. Pregnancy outcome and prepregnancy body mass index in 2459 glucose-tolerant Danish women. Am J Obstet Gynecol. 2003 Jul;189(1):239-44.
77. Rosenberg TJ, Garbers S, Lipkind H, Chiasson MA. Maternal obesity and diabetes as risk factors for adverse pregnancy outcomes: differences among 4 racial/ethnic groups. Am J Public Health. 2005 Sep;95(9):1545-51. DOI: 10.2105/ajph.2005.065680.
78. Cnattingius S, Villamor E, Johansson S, Edstedt Bonamy AK, Persson M, Wikstrom AK, et al. Maternal obesity and risk of preterm delivery. Jama. 2013 Jun 12;309(22):2362-70. DOI: 10.1001/jama.2013.6295.
79. Hendler I, Goldenberg RL, Mercer BM, Iams JD, Meis PJ, Moawad AH, et al. The Preterm Prediction Study: association between maternal body mass index and spontaneous and indicated preterm birth. Am J Obstet Gynecol. 2005 Mar;192(3):882-6. DOI: 10.1016/j.ajog.2004.09.021.
80. Galtier-Dereure F, Boegner C, Bringer J. Obesity and pregnancy: complications and cost. Am J Clin Nutr. 2000 May;71(5 Suppl):1242s-8s.
81. Doherty DA, Magann EF, Francis J, Morrison JC, Newnham JP. Pre-pregnancy body mass index and pregnancy outcomes. International journal of gynaecology and obstetrics: the official organ of the

International Federation of Gynaecology and Obstetrics. 2006 Dec;95(3):242-7. DOI: 10.1016/j.ijgo.2006.06.021.
82. Watson M, Howell S, Johnston T, Callaway L, Khor SL, Cornes S. Pre-pregnancy BMI: costs associated with maternal underweight and obesity in Queensland. Aust N Z J Obstet Gynaecol. 2013 Jun;53(3):243-9. DOI: 10.1111/ajo.12031.
83. Block SR, Watkins SM, Salemi JL, Rutkowski R, Tanner JP, Correia JA, et al. Maternal Pre-Pregnancy Body Mass Index and Risk of Selected Birth Defects: Evidence of a Dose–Response Relationship. Paediatric and Perinatal Epidemiology. [https://doi.org/10.1111/ppe.12084]. 2013 2013/11/01;27(6):521-31. DOI: https://doi.org/10.1111/ppe.12084.
84. Rasmussen SA, Chu SY, Kim SY, Schmid CH, Lau J. Maternal obesity and risk of neural tube defects: a metaanalysis. Am J Obstet Gynecol. 2008 Jun;198(6):611-9. DOI: 10.1016/j.ajog.2008.04.021.
85. Cai GJ, Sun XX, Zhang L, Hong Q. Association between maternal body mass index and congenital heart defects in offspring: a systematic review. Am J Obstet Gynecol. 2014 Aug;211(2):91-117. DOI: 10.1016/j.ajog.2014.03.028.
86. Persson M, Cnattingius S, Villamor E, Söderling J, Pasternak B, Stephansson O, et al. Risk of major congenital malformations in relation to maternal overweight and obesity severity: cohort study of 1.2 million singletons. BMJ. 2017;357:j2563. DOI: 10.1136/bmj.j2563.
87. Aagaard-Tillery KM, Flint Porter T, Malone FD, Nyberg DA, Collins J, Comstock CH, et al. Influence of maternal BMI on genetic sonography in the FaSTER trial. Prenat Diagn. 2010 Jan;30(1):14-22. DOI: 10.1002/pd.2399.
88. Dashe JS, McIntire DD, Twickler DM. Effect of maternal obesity on the ultrasound detection of anomalous fetuses. Obstet Gynecol. 2009 May;113(5):1001-7. DOI: 10.1097/AOG.0b013e3181a1d2f5.
89. Rosenberg TJ, Garbers S, Chavkin W, Chiasson MA. Prepregnancy weight and adverse perinatal outcomes in an ethnically diverse population. Obstetrics and gynecology. 2003 Nov;102(5 Pt 1):1022-7.
90. Freeman MD, Goodyear SM, Leith WM. A multistate population-based analysis of linked maternal and neonatal discharge records to identify risk factors for neonatal brachial plexus injury. Int J Gynaecol Obstet. 2017 Mar;136(3):331-6. DOI: 10.1002/ijgo.12059.
91. Athukorala C, Rumbold AR, Willson KJ, Crowther CA. The risk of adverse pregnancy outcomes in women who are overweight or obese. BMC pregnancy and childbirth. 2010;10:56. DOI: 10.1186/1471-2393-10-56.
92. Kitsantas P, Gaffney KF. Risk profiles for overweight/obesity among preschoolers. Early Hum Dev. 2010 Sep;86(9):563-8. DOI: 10.1016/j.earlhumdev.2010.07.006.
93. Rooney BL, Mathiason MA, Schauberger CW. Predictors of obesity in childhood, adolescence, and adulthood in a birth cohort. Matern Child Health J. 2011 Nov;15(8):1166-75. DOI: 10.1007/s10995-010-0689-1.
94. Jharap VV, Santos S, Steegers EA, Jaddoe VW, Gaillard R. Associations of maternal obesity and excessive weight gain during pregnancy with subcutaneous fat mass in infancy. Early Hum Dev. 2017 Mar 29;108:23-8. DOI: 10.1016/j.earlhumdev.2017.03.006.

95. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. Pediatrics. 2003 Mar;111(3):e221-6.
96. American College of Obstetrics and Gynecology. Macrosomia: ACOG Practice Bulletin, Number 216. Obstet Gynecol. 2020 Jan;135(1):e18-e35. DOI: 10.1097/aog.0000000000003606.
97. ACOG Committee on Obstetric Practice. ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. Obstetrics and gynecology. 2011 Jul;118(1):197-8. DOI: 10.1097/AOG.0b013e318227f06b.
98. Surkan PJ, Hsieh C-C, Johansson ALV, Dickman PW, Cnattingius S. Reasons for Increasing Trends in Large for Gestational Age Births. Obstetrics & Gynecology. 2004;104(4).
99. Ananth CV, Wen SW. Trends in fetal growth among singleton gestations in the United States and Canada, 1985 through 1998. Semin Perinatol. 2002 Aug;26(4):260-7. DOI: 10.1053/sper.2002.34772.
100. Ghosh RE, Berild JD, Sterrantino AF, Toledano MB, Hansell AL. Birth weight trends in England and Wales (1986-2012): babies are getting heavier. Arch Dis Child Fetal Neonatal Ed. 2018 May;103(3):F264-f70. DOI: 10.1136/archdischild-2016-311790.
101. Hadfield RM, Lain SJ, Simpson JM, Ford JB, Raynes-Greenow CH, Morris JM, et al. Are babies getting bigger? An analysis of birthweight trends in New South Wales, 1990–2005. Medical Journal of Australia. [https://doi.org/10.5694/j.1326-5377.2009.tb02420.x]. 2009 2009/03/01;190(6):312-5. DOI: https://doi.org/10.5694/j.1326-5377.2009.tb02420.x.
102. Weissmann-Brenner A, Simchen MJ, Zilberberg E, Kalter A, Weisz B, Achiron R, et al. Maternal and neonatal outcomes of large for gestational age pregnancies. Acta obstetricia et gynecologica Scandinavica. 2012 Jul;91(7):844-9. DOI: 10.1111/j.1600-0412.2012.01412.x.
103. Rosen H, Shmueli A, Ashwal E, Hiersch L, Yogev Y, Aviram A. Delivery outcomes of large-for-gestational-age newborns stratified by the presence or absence of gestational diabetes mellitus. International Journal of Gynecology & Obstetrics. [https://doi.org/10.1002/ijgo.12387]. 2018 2018/04/01;141(1):120-5. DOI: https://doi.org/10.1002/ijgo.12387.
104. Larkin JC, Speer PD, Simhan HN. A customized standard of large size for gestational age to predict intrapartum morbidity. American Journal of Obstetrics and Gynecology. 2011 2011/06/01/;204(6):499.e1-.e10. DOI: https://doi.org/10.1016/j.ajog.2011.02.068.
105. Modzelewski J, Pokropek A, Jakubiak-Proć M, Muzyka-Placzyńska K, Filipecka-Tyczka D, Kajdy A, et al. Large-for-gestational-age or macrosomia as a classifier for risk of adverse perinatal outcome: a retrospective cross-sectional study. J Matern Fetal Neonatal Med. 2021 Feb 18:1-8. DOI: 10.1080/14767058.2021.1887127.
106. Moschonis G, Grammatikaki E, Manios Y. Perinatal predictors of overweight at infancy and preschool childhood: the GENESIS study. Int J Obes (Lond). 2008 Jan;32(1):39-47. DOI: 10.1038/sj.ijo.0803764.

107. Dubois L, Girard M. Early determinants of overweight at 4.5 years in a population-based longitudinal study. Int J Obes (Lond). 2006 Apr;30(4):610-7. DOI: 10.1038/sj.ijo.0803141.
108. Johnsson IW, Haglund B, Ahlsson F, Gustafsson J. A high birth weight is associated with increased risk of type 2 diabetes and obesity. Pediatric Obesity. 2015;10(2):77-83. DOI: https://doi.org/10.1111/ijpo.230.
109. Barker DJ. Maternal nutrition, fetal nutrition, and disease in later life. Nutrition (Burbank, Los Angeles County, Calif). 1997 Sep;13(9):807-13. DOI: 10.1016/s0899-9007(97)00193-7.
110. Hanson MA, Gluckman PD, Ma RC, Matzen P, Biesma RG. Early life opportunities for prevention of diabetes in low and middle income countries. BMC Public Health. 2012 Nov 23;12:1025. DOI: 10.1186/1471-2458-12-1025.
111. Tam WH, Ma RC, Yang X, Li AM, Ko GT, Kong AP, et al. Glucose intolerance and cardiometabolic risk in adolescents exposed to maternal gestational diabetes: a 15-year follow-up study. Diabetes Care. 2010 Jun;33(6):1382-4. DOI: 10.2337/dc09-2343.
112. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. Am J Obstet Gynecol. 2006 Oct;195(4):1100-3. DOI: 10.1016/j.ajog.2006.06.014.
113. Sparks JW. Human intrauterine growth and nutrient accretion. Semin Perinatol. 1984 Apr;8(2):74-93.
114. Heerwagen MJ, Miller MR, Barbour LA, Friedman JE. Maternal obesity and fetal metabolic programming: a fertile epigenetic soil. Am J Physiol Regul Integr Comp Physiol. [Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't
Review]. 2010 Sep;299(3):R711-22. doi: 10.1152/ajpregu.00310.2010. Epub 2010 Jul 14.
115. Budge H, Gnanalingham MG, Gardner DS, Mostyn A, Stephenson T, Symonds ME. Maternal nutritional programming of fetal adipose tissue development: long-term consequences for later obesity. Birth defects research Part C, Embryo today : reviews. 2005 Sep;75(3):193-9. DOI: 10.1002/bdrc.20044.
116. Symonds ME, Pope M, Sharkey D, Budge H. Adipose tissue and fetal programming. Diabetologia. 2012 Jun;55(6):1597-606. DOI: 10.1007/s00125-012-2505-5.
117. Catalano PM, Tyzbir ED, Allen SR, McBean JH, McAuliffe TL. Evaluation of fetal growth by estimation of neonatal body composition. Obstet Gynecol. 1992 Jan;79(1):46-50.
118. Grivell RM, Yelland LN, Deussen A, Crowther CA, Dodd JM. Antenatal dietary and lifestyle advice for women who are overweight or obese and the effect on fetal growth and adiposity: the LIMIT randomised trial. BJOG. [Randomized Controlled Trial
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. 2016 Jan;123(2):233-43. doi: 10.1111/471-0528.13777.
119. Larciprete G, Valensise H, Vasapollo B, Novelli GP, Parretti E, Altomare F, et al. Fetal subcutaneous tissue thickness (SCTT) in healthy and

gestational diabetic pregnancies. Ultrasound Obstet Gynecol. 2003 Dec;22(6):591-7. DOI: 10.1002/uog.926.
120. Bernstein IM, Catalano PM. Ultrasonographic estimation of fetal body composition for children of diabetic mothers. Invest Radiol. 1991 Aug;26(8):722-6.
121. Gardeil F, Greene R, Stuart B, Turner MJ. Subcutaneous fat in the fetal abdomen as a predictor of growth restriction. Obstet Gynecol. 1999 Aug;94(2):209-12.
122. Bernstein IM, Goran MI, Amini SB, Catalano PM. Differential growth of fetal tissues during the second half of pregnancy. Am J Obstet Gynecol. 1997 Jan;176(1 Pt 1):28-32.
123. Hill LM, Guzick D, Boyles D, Merolillo C, Ballone A, Gmiter P. Subcutaneous tissue thickness cannot be used to distinguish abnormalities of fetal growth. Obstet Gynecol. 1992 Aug;80(2):268-71.
124. Catalano PM, Farrell K, Thomas A, Huston-Presley L, Mencin P, de Mouzon SH, et al. Perinatal risk factors for childhood obesity and metabolic dysregulation. Am J Clin Nutr. 2009 Nov;90(5):1303-13. DOI: 10.3945/ajcn.2008.27416.
125. Moyer-Mileur LJ, Slater H, Thomson JA, Mihalopoulos N, Byrne J, Varner MW. Newborn adiposity measured by plethysmography is not predicted by late gestation two-dimensional ultrasound measures of fetal growth. J Nutr. [Research Support, N.I.H., Extramural
Validation Studies]. 2009 Sep;139(9):1772-8. doi: 10.3945/jn.109.109058. Epub 2009 Jul 29.
126. Roelants JA, de Jonge RC, Steegers-Theunissen RP, Reiss IK, Joosten KF, Vermeulen MJ. Prenatal markers of neonatal fat mass: A systematic review. Clinical nutrition (Edinburgh, Scotland). 2016 Oct;35(5):995-1007. DOI: 10.1016/j.clnu.2015.09.003.
127. O'Connor C, Doolan A, O'Higgins A, Segurado R, Sheridan-Pereiraet M, Turner MJ, et al. Fetal subcutaneous tissue measurements in pregnancy as a predictor of neonatal total body composition. Prenat Diagn. 2014 Oct;34(10):952-5. DOI: 10.1002/pd.4400.
128. Moore GS, Allshouse AA, Fisher BM, Kahn BF, Hernandez TL, Reece MS, et al. Can Fetal Limb Soft Tissue Measurements in the Third Trimester Predict Neonatal Adiposity? J Ultrasound Med. 2016 Sep;35(9):1915-24. DOI: 10.7863/ultra.15.06028.
129. Buhling KJ, Doll I, Siebert G, Catalano PM. Relationship between sonographically estimated fetal subcutaneous adipose tissue measurements and neonatal skinfold measurements. Ultrasound Obstet Gynecol. 2012 May;39(5):558-62. DOI: 10.1002/uog.10092.
130. Hull HR, Dinger MK, Knehans AW, Thompson DM, Fields DA. Impact of maternal body mass index on neonate birthweight and body composition. Am J Obstet Gynecol. 2008 Apr;198(4):416 e1-6. DOI: 10.1016/j.ajog.2007.10.796.
131. Starling AP, Brinton JT, Glueck DH, Shapiro AL, Harrod CS, Lynch AM, et al. Associations of maternal BMI and gestational weight gain with neonatal adiposity in the Healthy Start study. The American journal of clinical nutrition. 2015 Feb;101(2):302-9. DOI: 10.3945/ajcn.114.094946.

132. Madendag Y, Aksoy U, Col Madendag I, Aksoy H. Fetal front-abdominal wall thickness in the second trimester as a predictor of abnormal fetal growth. J Matern Fetal Neonatal Med. 2020 Mar 24:1-7. DOI: 10.1080/14767058.2020.1743666.
133. Caradeux J, Eixarch E, Mazarico E, Basuki TR, Gratacós E, Figueras F. Second- to Third-Trimester Longitudinal Growth Assessment for the Prediction of Largeness for Gestational Age and Macrosomia in an Unselected Population. Fetal diagnosis and therapy. 2018;43(4):284-90. DOI: 10.1159/000477460.
134. Pedersen J. The Pregnant Diabetic and Her Newborn: Problems and Management. Baltimore: Williams and Wilkins; 1967.
135. Pedersen J. Weight and length at birth of infants of diabetic mothers. Acta endocrinologica. 1954 Aug;16(4):330-42.
136. Metzger BE, Gabbe SG, Persson B, Buchanan TA, Catalano PA, Damm P, et al. International association of diabetes and pregnancy study groups recommendations on the diagnosis and classification of hyperglycemia in pregnancy. Diabetes Care. 2010 Mar;33(3):676-82. DOI: 10.2337/dc09-1848.
137. World Health Organisation. Diagnostic Criteria and Classification of Hyperglycaemia First Detected in Pregnancy. Switzerland; 2013.
138. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009 Jan;32 Suppl 1:S62-7. DOI: 10.2337/dc09-S062.
139. O'Sullivan JB. Establishing criteria for gestational diabetes. Diabetes care. 1980;3(3):437-9.
140. American Diabetes Association. Summary and Recommendations. Diabetes Care. 1980;3(3):499-501.
141. Crowther CA, Hiller JE, Moss JR, McPhee AJ, Jeffries WS, Robinson JS. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. N Engl J Med. 2005 Jun 16;352(24):2477-86. DOI: 10.1056/NEJMoa042973.
142. Landon MB, Spong CY, Thom E, Carpenter MW, Ramin SM, Casey B, et al. A multicenter, randomized trial of treatment for mild gestational diabetes. N Engl J Med. 2009 Oct 01;361(14):1339-48. DOI: 10.1056/NEJMoa0902430.
143. Metzger BE, Lowe LP, Dyer AR, Trimble ER, Chaovarindr U, Coustan DR, et al. Hyperglycemia and adverse pregnancy outcomes. The New England journal of medicine. 2008 May 8;358(19):1991-2002. DOI: 10.1056/NEJMoa0707943.
144. World Health Organization. Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus: World Health Organization; 1999.
145. ACOG Practice Bulletin No. 190: Gestational Diabetes Mellitus. Obstet Gynecol. 2018 Feb;131(2):e49-e64. DOI: 10.1097/aog.0000000000002501.
146. National Institute for Health and Care Excellence (NICE). Gestational diabetes: risk assessment, testing, diagnosis and management. 2021.
147. Ivers NM, Jiang M, Alloo J, Singer A, Ngui D, Casey CG, et al. Diabetes Canada 2018 clinical practice guidelines: Key messages for family

physicians caring for patients living with type 2 diabetes. Canadian family physician Medecin de famille canadien. 2019;65(1):14-24. Cited in: PubMed.
148. Blank A, Grave GD, Metzger BE. Effects of gestational diabetes on perinatal morbidity reassessed. Report of the International Workshop on Adverse Perinatal Outcomes of Gestational Diabetes Mellitus, December 3-4, 1992. Diabetes Care. 1995 Jan;18(1):127-9.
149. Metzger BE, Buchanan TA, Coustan DR, de Leiva A, Dunger DB, Hadden DR, et al. Summary and recommendations of the Fifth International Workshop-Conference on Gestational Diabetes Mellitus. Diabetes Care. 2007 Jul;30 Suppl 2:S251-60. DOI: 10.2337/dc07-s225.
150. Nankervis A, McIntyre H, Moses R, Ross G, Callaway L, Porter C, et al. ADIPS consensus guidelines for the testing and diagnosis of gestational diabetes mellitus in Australia. Modified June. 2014.
151. Hillier TA, Pedula KL, Ogasawara KK, Vesco KK, Oshiro CES, Lubarsky SL, et al. A Pragmatic, Randomized Clinical Trial of Gestational Diabetes Screening. New England Journal of Medicine. 2021 2021/03/11;384(10):895-904. DOI: 10.1056/NEJMoa2026028.
152. Davis EM, Abebe KZ, Simhan HN, Catalano P, Costacou T, Comer D, et al. Perinatal Outcomes of Two Screening Strategies for Gestational Diabetes Mellitus: A Randomized Controlled Trial. Obstet Gynecol. 2021 Jul 1;138(1):6-15. DOI: 10.1097/aog.0000000000004431.
153. Berger H, Gagnon R, Sermer M, Basso M, Bos H, Brown RN, et al. Diabetes in Pregnancy. Journal of obstetrics and gynaecology Canada : JOGC = Journal d'obstetrique et gynecologie du Canada : JOGC. 2016 Jul;38(7):667-79.e1. DOI: 10.1016/j.jogc.2016.04.002.
154. O'Sullivan EP, Avalos G, O'Reilly M, Dennedy MC, Gaffney G, Dunne F. Atlantic Diabetes in Pregnancy (DIP): the prevalence and outcomes of gestational diabetes mellitus using new diagnostic criteria. Diabetologia. 2011 Jul;54(7):1670-5. DOI: 10.1007/s00125-011-2150-4.
155. Farrar D, Simmonds M, Bryant M, Sheldon TA, Tuffnell D, Golder S, et al. Hyperglycaemia and risk of adverse perinatal outcomes: systematic review and meta-analysis. Bmj. 2016 Sep 13;354:i4694. DOI: 10.1136/bmj.i4694.
156. Shah BR, Sharifi F. Perinatal outcomes for untreated women with gestational diabetes by IADPSG criteria: a population-based study. Bjog. 2020 Jan;127(1):116-22. DOI: 10.1111/1471-0528.15964.
157. Shand AW, Bell JC, McElduff A, Morris J, Roberts CL. Outcomes of pregnancies in women with pre-gestational diabetes mellitus and gestational diabetes mellitus; a population-based study in New South Wales, Australia, 1998-2002. Diabetic medicine : a journal of the British Diabetic Association. 2008 Jun;25(6):708-15. DOI: 10.1111/j.1464-5491.2008.02431.x.
158. Silverman BL, Metzger BE, Cho NH, Loeb CA. Impaired glucose tolerance in adolescent offspring of diabetic mothers. Relationship to fetal hyperinsulinism. Diabetes Care. 1995 May;18(5):611-7.
159. Wright CS, Rifas-Shiman SL, Rich-Edwards JW, Taveras EM, Gillman MW, Oken E. Intrauterine Exposure to Gestational Diabetes, Child Adiposity,

and Blood Pressure. Am J Hypertens. 2009 Feb;22(2):215-20. DOI: 10.1038/ajh.2008.326.
160. Silverman BL, Rizzo T, Green OC, Cho NH, Winter RJ, Ogata ES, et al. Long-term prospective evaluation of offspring of diabetic mothers. Diabetes. 1991 Dec;40 Suppl 2:121-5. DOI: 10.2337/diab.40.2.s121.
161. Gillman MW, Oakey H, Baghurst PA, Volkmer RE, Robinson JS, Crowther CA. Effect of treatment of gestational diabetes mellitus on obesity in the next generation. Diabetes Care. 2010 May;33(5):964-8. DOI: 10.2337/dc09-1810.
162. Kawasaki M, Arata N, Miyazaki C, Mori R, Kikuchi T, Ogawa Y, et al. Obesity and abnormal glucose tolerance in offspring of diabetic mothers: A systematic review and meta-analysis. PLoS One. 2018;13(1):e0190676. DOI: 10.1371/journal.pone.0190676.
163. Laafira A, White SW, Griffin CJ, Graham D. Impact of the new IADPSG gestational diabetes diagnostic criteria on pregnancy outcomes in Western Australia. Aust N Z J Obstet Gynaecol. 2016 Feb;56(1):36-41. DOI: 10.1111/ajo.12394.
164. Svare JA, Hansen BB, Molsted-Pedersen L. Perinatal complications in women with gestational diabetes mellitus. Acta obstetricia et gynecologica Scandinavica. 2001 Oct;80(10):899-904.
165. Harmon KA, Gerard L, Jensen DR, Kealey EH, Hernandez TL, Reece MS, et al. Continuous glucose profiles in obese and normal-weight pregnant women on a controlled diet: metabolic determinants of fetal growth. Diabetes Care. 2011 Oct;34(10):2198-204. DOI: 10.2337/dc11-0723.
166. Langer O, Yogev Y, Xenakis EM, Brustman L. Overweight and obese in gestational diabetes: the impact on pregnancy outcome. Am J Obstet Gynecol. 2005 Jun;192(6):1768-76. DOI: 10.1016/j.ajog.2004.12.049.
167. HAPO Study Cooperative Research Group. Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) Study: associations with maternal body mass index. BJOG. [Multicenter Study
Research Support, N.I.H., Extramural
Research Support, Non-U.S. Gov't]. 2010 Apr;117(5):575-84. doi: 10.1111/j.471-0528.2009.02486.x. Epub 2010 Jan 20.
168. Catalano PM, McIntyre HD, Cruickshank JK, McCance DR, Dyer AR, Metzger BE, et al. The hyperglycemia and adverse pregnancy outcome study: associations of GDM and obesity with pregnancy outcomes. Diabetes Care. 2012 Apr;35(4):780-6. DOI: 10.2337/dc11-1790.
169. Shoelson SE, Herrero L, Naaz A. Obesity, Inflammation, and Insulin Resistance. Gastroenterology. 2007 2007/05/01/;132(6):2169-80. DOI: https://doi.org/10.1053/j.gastro.2007.03.059.
170. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. American Journal of Physiology-Endocrinology and Metabolism. 2001 2001/05/01;280(5):E745-E51. DOI: 10.1152/ajpendo.2001.280.5.E745.
171. Howell KR, Powell TL. Effects of maternal obesity on placental function and fetal development. Reproduction (Cambridge, England). 2017;153(3):R97-R108. DOI: 10.1530/REP-16-0495. Cited in: PubMed.

172. Elksne K, Jurka A, Rezeberga D. TNF-[alpha] And IL-8 As Prognostic Markers of Birth Outcome in Overweight and Non-Overweight Pregnant Women. Acta Chirurgica Latviensis. 2013 2013 2013-09-20;13(1):45-n/a. DOI: http://dx.doi.org/10.2478/chilat-2013-0009. Cited in: ProQuest One Academic.
173. Mestan K, Ouyang F, Matoba N, Pearson C, Ortiz K, Wang X. Maternal obesity, diabetes mellitus and cord blood biomarkers in large-for-gestational age infants. Journal of Pediatric Biochemistry. 2010;1:217-24. DOI: 10.3233/JPB-2010-0023.
174. Christian LM, Porter K. Longitudinal changes in serum proinflammatory markers across pregnancy and postpartum: effects of maternal body mass index. Cytokine. 2014;70(2):134-40.
175. Aye IL, Lager S, Ramirez VI, Gaccioli F, Dudley DJ, Jansson T, et al. Increasing maternal body mass index is associated with systemic inflammation in the mother and the activation of distinct placental inflammatory pathways. Biology of reproduction. 2014;90(6):129, 1-9.
176. Stone RA, Silvis A, Jude D, Chaffin D. Increasing body mass index exacerbates inflammation in obese gravidas. Obstetrics & Gynecology. 2014;123:81S.
177. Saucedo R, Valencia J, Moreno-González L, Peña-Cano M, Aranda-Martínez A, García Y, et al. Maternal serum adipokines and inflammatory markers at late gestation and newborn weight in mothers with and without gestational diabetes mellitus. Ginekol Pol. 2021 2021-04-20;0(0). DOI: 10.5603/GP.a2021.0083.
178. Knopp RH, Bergelin RO, Wahl PW, Walden CE. Relationships of Infant Birth Size to Maternal Lipoproteins, Apoproteins, Fuels, Hormones, Clinical Chemistries, and Body Weight at 36 Weeks Gestation. Diabetes. 1985;34(Supplement_2):71-7. DOI: 10.2337/diab.34.2.S71.
179. Schaefer-Graf UM, Graf K, Kulbacka I, Kjos SL, Dudenhausen J, Vetter K, et al. Maternal Lipids as Strong Determinants of Fetal Environment and Growth in Pregnancies With Gestational Diabetes Mellitus. Diabetes Care. 2008;31(9):1858-63. DOI: 10.2337/dc08-0039.
180. Son GH, Kwon JY, Kim YH, Park YW. Maternal serum triglycerides as predictive factors for large-for-gestational age newborns in women with gestational diabetes mellitus. Acta obstetricia et gynecologica Scandinavica. 2010 May;89(5):700-4. DOI: 10.3109/00016341003605677.
181. Di Cianni G, Miccoli R, Volpe L, Lencioni C, Ghio A, Giovannitti MG, et al. Maternal triglyceride levels and newborn weight in pregnant women with normal glucose tolerance. Diabetic Medicine. [https://doi.org/10.1111/j.1464-5491.2004.01336.x]. 2005 2005/01/01;22(1):21-5. DOI: https://doi.org/10.1111/j.1464-5491.2004.01336.x.
182. Sovio U, Murphy HR, Smith GC. Accelerated Fetal Growth Prior to Diagnosis of Gestational Diabetes Mellitus: A Prospective Cohort Study of Nulliparous Women. Diabetes Care. 2016 Jun;39(6):982-7. DOI: 10.2337/dc16-0160.
183. Schaefer-Graf UM, Kjos SL, Kilavuz O, Plagemann A, Brauer M, Dudenhausen JW, et al. Determinants of fetal growth at different periods

of pregnancies complicated by gestational diabetes mellitus or impaired glucose tolerance. Diabetes Care. 2003 Jan;26(1):193-8.
184. Ay L, Kruithof CJ, Bakker R, Steegers EA, Witteman JC, Moll HA, et al. Maternal anthropometrics are associated with fetal size in different periods of pregnancy and at birth. The Generation R Study. Bjog. 2009 Jun;116(7):953-63. DOI: 10.1111/j.1471-0528.2009.02143.x.
185. Zhang C, Hediger ML, Albert PS, Grewal J, Sciscione A, Grobman WA, et al. Association of Maternal Obesity With Longitudinal Ultrasonographic Measures of Fetal Growth: Findings From the NICHD Fetal Growth Studies-Singletons. JAMA pediatrics. 2017 Nov 13. DOI: 10.1001/jamapediatrics.2017.3785.
186. Schaefer-Graf UM, Heuer R, Kilavuz O, Pandura A, Henrich W, Vetter K. Maternal obesity not maternal glucose values correlates best with high rates of fetal macrosomia in pregnancies complicated by gestational diabetes. J Perinat Med. 2002;30(4):313-21. DOI: 10.1515/jpm.2002.046.
187. Black MH, Sacks DA, Xiang AH, Lawrence JM. The relative contribution of prepregnancy overweight and obesity, gestational weight gain, and IADPSG-defined gestational diabetes mellitus to fetal overgrowth. Diabetes Care. 2013 Jan;36(1):56-62. DOI: 10.2337/dc12-0741.
188. Dodd JM, Turnbull D, McPhee AJ, Deussen AR, Grivell RM, Yelland LN, et al. Antenatal lifestyle advice for women who are overweight or obese: LIMIT randomised trial. BMJ (Clinical research ed). [Multicenter Study
Randomized Controlled Trial
Research Support, Non-U.S. Gov't]. 2014 Feb 10;348:g1285. DOI: https://dx.doi.org/10.1136/bmj.g1285. Cited in: MEDLINE.
189. Poston L, Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, et al. Effect of a behavioural intervention in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. Lancet Diabetes Endocrinol. 2015 Oct;3(10):767-77. DOI: 10.1016/S2213-8587(15)00227-2.
190. Dodd JM, Cramp C, Sui Z, Yelland LN, Deussen AR, Grivell RM, et al. The effects of antenatal dietary and lifestyle advice for women who are overweight or obese on maternal diet and physical activity: the LIMIT randomised trial. BMC medicine. 2014 Oct 13;12:161. DOI: 10.1186/s12916-014-0161-y.
191. i-WIP Collaborative Group. Effect of diet and physical activity based interventions in pregnancy on gestational weight gain and pregnancy outcomes: meta-analysis of individual participant data from randomised trials. Bmj. 2017 Jul 19;358:j3119. DOI: 10.1136/bmj.j3119.
192. Rowan JA, Hague WM, Gao W, Battin MR, Moore MP. Metformin versus insulin for the treatment of gestational diabetes. N Engl J Med. 2008 May 08;358(19):2003-15. DOI: 10.1056/NEJMoa0707193.
193. Dodd JM, Louise J, Deussen AR, Grivell RM, Dekker G, McPhee AJ, et al. Effect of metformin in addition to dietary and lifestyle advice for pregnant women who are overweight or obese: the GRoW randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2018 Dec 4. DOI: 10.1016/s2213-8587(18)30310-3.
194. Chiswick C, Reynolds RM, Denison F, Drake AJ, Forbes S, Newby DE, et al. Effect of metformin on maternal and fetal outcomes in obese pregnant women (EMPOWaR): a randomised, double-blind, placebo-controlled

trial. Lancet Diabetes Endocrinol. 2015 Oct;3(10):778-86. DOI: 10.1016/S2213-8587(15)00219-3.
195. Syngelaki A, Nicolaides KH, Balani J, Hyer S, Akolekar R, Kotecha R, et al. Metformin versus Placebo in Obese Pregnant Women without Diabetes Mellitus. N Engl J Med. 2016 Feb 04;374(5):434-43. DOI: 10.1056/NEJMoa1509819.
196. Nascimento IBD, Sales WB, Dienstmann G, Souza MLR, Fleig R, Silva JC. Metformin for prevention of cesarean delivery and large-for-gestational-age newborns in non-diabetic obese pregnant women: a randomized clinical trial. Archives of endocrinology and metabolism. 2020 May-Jun;64(3):290-7. DOI: 10.20945/2359-3997000000251.
197. Dienstmann G, Nascimento IBD, Sales WB, Leite Ramos de Souza M, Silva GDD, de Oliveira LC, et al. No effect of a low dose of metformin on the lipid profile, body mass index and weight gain in pregnant women with obesity: A randomized trial. Obesity research & clinical practice. 2020 Nov-Dec;14(6):561-5. DOI: 10.1016/j.orcp.2020.09.005.
198. Sales WB, Nascimento IBD, Dienstmann G, Souza MLR, Silva GDD, Silva JC. Effectiveness of Metformin in the Prevention of Gestational Diabetes Mellitus in Obese Pregnant Women. Revista brasileira de ginecologia e obstetricia : revista da Federacao Brasileira das Sociedades de Ginecologia e Obstetricia. 2018 Apr;40(4):180-7. DOI: 10.1055/s-0038-1642632.
199. Dodd JM. Dietary and lifestyle advice for pregnant women who are overweight or obese: the LIMIT randomized trial. Ann Nutr Metab. 2014;64(3-4):197-202. DOI: 10.1159/000365018.
200. SA Health. South Australian Perinatal Practice Guidelines: Normal Pregnancy, Labour and Puerperium Management. In: Health S, editor. Adelaide, South Australia: SA Health, Government of South Australia; 2015.
201. Dodd JM, Deussen AR, Louise J. A Randomised Trial to Optimise Gestational Weight Gain and Improve Maternal and Infant Health Outcomes through Antenatal Dietary, Lifestyle and Exercise Advice: The OPTIMISE Randomised Trial. Nutrients. 2019 Dec 2;11(12). DOI: 10.3390/nu11122911.
202. SA Health. South Australian Perinatal Practice Guidelines: Diabetes Mellitus and Abnormal Glucose Tolerance. In: Health S, editor. Adelaide, South Australia: SA Health, Government of Australia; 2013.
203. Australian Diabetes in Pregnancy Society. ADIPS Consensus Guidelines for the Testing and Diagnosis of Hyperglycaemia in Pregnancy in Australia and New Zealand; 2014 November 2014.
204. Dodd JM, Louise J, Deussen AR, Grivell RM, Dekker G, McPhee AJ, et al. Effect of metformin in addition to dietary and lifestyle advice for pregnant women who are overweight or obese: the GRoW randomised, double-blind, placebo-controlled trial. Lancet Diabetes Endocrinol. 2019 Jan;7(1):15-24. DOI: 10.1016/s2213-8587(18)30310-3.
205. Department of Health. The Australian Guide to Healthy Eating. Australian Government; 2013 [updated 28 October 2013; cited 2020 14 December 2020]. Available from:

https://www1.health.gov.au/internet/publications/publishing.nsf/Content/nhsc-guidelines~aus-guide-healthy-eating
206. Dodd JM, Grivell RM, Deussen AR, Dekker G, Louise J, Hague W. Metformin and dietary advice to improve insulin sensitivity and promote gestational restriction of weight among pregnant women who are overweight or obese: the GRoW Randomised Trial. BMC pregnancy and childbirth. 2016;16. DOI: 10.1186/s12884-016-1161-z.
207. Dodd JM, Deussen AR, Louise J. Optimising gestational weight gain and improving maternal and infant health outcomes through antenatal dietary, lifestyle and physical activity advice: the OPTIMISE randomised controlled trial protocol. BMJ Open. 2018 Feb 20;8(2):e019583. DOI: 10.1136/bmjopen-2017-019583.
208. Dodd J, Deussen A. Optimising gestational weight gain and improving maternal and infant health outcomes through antenatal dietary, lifestyle and exercise advice: the OPTIMISE randomised trial. 2015;ACTRN: 12614000583640.
209. Australian Society for Ultrasound in Medicine. Promoting Excellence in Ultrasound-Policy D7: Statement on Normal Ultrasonographic Fetal Measurements.: Crows Nest; 2007.
210. Bethune M, Alibrahim E, Davies B, Yong E. A pictorial guide for the second trimester ultrasound. Australasian journal of ultrasound in medicine. 2013 Aug;16(3):98-113. DOI: 10.1002/j.2205-0140.2013.tb00106.x.
211. Australian Society for Ultrasound in Medicine. Normal Ultrasonic Fetal Measurements Standard. 2018.
212. Hadlock FP, Harrist RB, Carpenter RJ, Deter RL, Park SK. Sonographic estimation of fetal weight. The value of femur length in addition to head and abdomen measurements. Radiology. 1984 Feb;150(2):535-40. DOI: 10.1148/radiology.150.2.6691115.
213. Westerway SC, Davison A, Cowell S. Ultrasonic fetal measurements: new Australian standards for the new millennium. Aust N Z J Obstet Gynaecol. 2000 Aug;40(3):297-302. DOI: 10.1111/j.1479-828x.2000.tb03338.x.
214. Owen P, Donnet ML, Ogston SA, Christie AD, Howie PW, Patel NB. Standards for ultrasound fetal growth velocity. British journal of obstetrics and gynaecology. 1996 Jan;103(1):60-9. DOI: 10.1111/j.1471-0528.1996.tb09516.x.
215. Higgins MF, Russell NM, Mulcahy CH, Coffey M, Foley ME, McAuliffe FM. Fetal anterior abdominal wall thickness in diabetic pregnancy. Eur J Obstet Gynecol Reprod Biol. 2008 Sep;140(1):43-7. DOI: 10.1016/j.ejogrb.2008.02.021.
216. Lopes KRM, Souza ASR, Figueiroa JN, Alves JGB. Correlation between pre-pregnancy body mass index and maternal visceral adiposity with fetal biometry during the second trimester. International Journal of Gynecology & Obstetrics. [https://doi.org/10.1002/ijgo.12202]. 2017 2017/08/01;138(2):133-7. DOI: https://doi.org/10.1002/ijgo.12202.
217. Royston P, Altman DG. Regression Using Fractional Polynomials of Continuous Covariates: Parsimonious Parametric Modelling. Journal of the Royal Statistical Society: Series C (Applied Statistics). [https://doi.org/10.2307/2986270]. 1994 1994/09/01;43(3):429-53. DOI: https://doi.org/10.2307/2986270.

218. Ambler, G. modified by Benner, A. . Mfp: Multivariable Fractional Polynomials. R package version 1.5.2. 2015. Available from: https://CRAN.R-project.org/package=mfp
219. Tanaka K, Matsushima M, Izawa T, Furukawa S, Kobayashi Y, Iwashita M. Influence of maternal obesity on fetal growth at different periods of pregnancies with normal glucose tolerance. The journal of obstetrics and gynaecology research. 2018 Apr;44(4):691-6. DOI: 10.1111/jog.13575.
220. Poston L, Bell R, Croker H, Flynn AC, Godfrey KM, Goff L, et al. Effect of a behavioural intervention in obese pregnant women (the UPBEAT study): a multicentre, randomised controlled trial. Lancet Diabetes Endocrinol. 2015 Oct;3(10):767-77. DOI: 10.1016/s2213-8587(15)00227-2.
221. Renault KM, Norgaard K, Nilas L, Carlsen EM, Cortes D, Pryds O, et al. The Treatment of Obese Pregnant Women (TOP) study: a randomized controlled trial of the effect of physical activity intervention assessed by pedometer with or without dietary intervention in obese pregnant women. Am J Obstet Gynecol. 2014 Feb;210(2):134.e1-9. DOI: 10.1016/j.ajog.2013.09.029.
222. Modi N, Murgasova D, Ruager-Martin R, Thomas EL, Hyde MJ, Gale C, et al. The Influence of Maternal Body Mass Index on Infant Adiposity and Hepatic Lipid Content. Pediatric Research. 2011 2011/09/01;70(3):287-91. DOI: 10.1203/PDR.0b013e318225f9b1.
223. Catalano PM, Presley L, Minium J, Hauguel-de Mouzon S. Fetuses of obese mothers develop insulin resistance in utero. Diabetes Care. 2009 Jun;32(6):1076-80. DOI: 10.2337/dc08-2077.
224. Merklin RJ. Growth and distribution of human fetal brown fat. The Anatomical record. 1974 Mar;178(3):637-45. DOI: 10.1002/ar.1091780311.
225. Kehl RJ, Krew MA, Thomas A, Catalano PAA. Fetal Growth and Body Composition in Infants of Women with Diabetes Mellitus During Pregnancy. Journal of Maternal-Fetal Medicine. 1996 1996/01/01;5(5):273-80. DOI: 10.3109/14767059609025434.
226. Parretti E, Carignani L, Cioni R, Bartoli E, Borri P, La Torre P, et al. Sonographic evaluation of fetal growth and body composition in women with different degrees of normal glucose metabolism. Diabetes Care. 2003 Oct;26(10):2741-8. DOI: 10.2337/diacare.26.10.2741.
227. Bethune M, Bell R. Evaluation of the measurement of the fetal fat layer, interventricular septum and abdominal circumference percentile in the prediction of macrosomia in pregnancies affected by gestational diabetes. Ultrasound Obstet Gynecol. 2003 Dec;22(6):586-90. DOI: 10.1002/uog.885.
228. Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. The mediating effects of gestational diabetes on fetal growth and adiposity in women who are overweight and obese: secondary analysis of the LIMIT randomised trial. Bjog. 2018 May 21. DOI: 10.1111/1471-0528.15288.
229. Catalano PM. The impact of gestational diabetes and maternal obesity on the mother and her offspring. J Dev Orig Health Dis. 2010 Aug;1(4):208-15. DOI: 10.1017/S2040174410000115.
230. Valeri L, Vanderweele TJ. Mediation analysis allowing for exposure-mediator interactions and causal interpretation: theoretical assumptions

and implementation with SAS and SPSS macros. Psychological methods. 2013 Jun;18(2):137-50. DOI: 10.1037/a0031034.
231. Liu AE, R. Dunn, G. VanderWeele, T. Valeri, L. paramed: A command to perform causal mediation analysis using parametric models. 2014.
232. Gaudet L, Ferraro ZM, Wen SW, Walker M. Maternal obesity and occurrence of fetal macrosomia: a systematic review and meta-analysis. BioMed research international. 2014;2014:640291. DOI: 10.1155/2014/640291.
233. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci. 2013 Apr 20;9(2):191-200. DOI: 10.5114/aoms.2013.33181.
234. Vrijkotte TG, Algera SJ, Brouwer IA, van Eijsden M, Twickler MB. Maternal triglyceride levels during early pregnancy are associated with birth weight and postnatal growth. The Journal of pediatrics. 2011 Nov;159(5):736-42.e1. DOI: 10.1016/j.jpeds.2011.05.001.
235. Tsai PJ, Davis J, Bryant-Greenwood G. Systemic and placental leptin and its receptors in pregnancies associated with obesity. Reprod Sci. 2015 Feb;22(2):189-97. DOI: 10.1177/1933719114537718.
236. Karakosta P, Chatzi L, Plana E, Margioris A, Castanas E, Kogevinas M. Leptin levels in cord blood and anthropometric measures at birth: a systematic review and meta-analysis. Paediatric and perinatal epidemiology. 2011 Mar;25(2):150-63. DOI: 10.1111/j.1365-3016.2010.01163.x.
237. Ategbo JM, Grissa O, Yessoufou A, Hichami A, Dramane KL, Moutairou K, et al. Modulation of adipokines and cytokines in gestational diabetes and macrosomia. The Journal of clinical endocrinology and metabolism. 2006 Oct;91(10):4137-43. DOI: 10.1210/jc.2006-0980.
238. Akiba Y, Ikenoue S, Endo T, Kasuga Y, Ochiai D, Miyakoshi K, et al. Differences in fetal fractional limb volume changes in normal and gestational diabetic pregnancies: an exploratory observational study. BJOG: An International Journal of Obstetrics & Gynaecology. [https://doi.org/10.1111/1471-0528.16265]. 2021 2021/01/01;128(2):329-35. DOI: https://doi.org/10.1111/1471-0528.16265.
239. Mulder EJH, Koopman CM, Vermunt JK, de Valk HW, Visser GHA. Fetal growth trajectories in Type-1 diabetic pregnancy. Ultrasound in Obstetrics & Gynecology. [https://doi.org/10.1002/uog.7700]. 2010 2010/12/01;36(6):735-42. DOI: https://doi.org/10.1002/uog.7700.
240. Hammoud NM, Visser GHA, Peters SAE, Graatsma EM, Pistorius L, de Valk HW. Fetal growth profiles of macrosomic and non-macrosomic infants of women with pregestational or gestational diabetes. Ultrasound in Obstetrics & Gynecology. [https://doi.org/10.1002/uog.11221]. 2013 2013/04/01;41(4):390-7. DOI: https://doi.org/10.1002/uog.11221.
241. Lee BH, Park TC, Lee HJ. Association between fetal abdominal circumference and birthweight in maternal hyperglycemia. Acta obstetricia et gynecologica Scandinavica. [https://doi.org/10.1111/aogs.12420]. 2014 2014/08/01;93(8):786-93. DOI: https://doi.org/10.1111/aogs.12420.

242. Beeby PJ, Bhutap T, Taylor LK. New South Wales population-based birthweight percentile charts. Journal of paediatrics and child health. 1996 Dec;32(6):512-8.
243. Australian Bureau of Statistics. Socio-Economic Indexes for Areas (SEIFA). Australian Capital Territory: Australian Bureau of Statistics; 2011.
244. Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements--a prospective study. Am J Obstet Gynecol. 1985 Feb 01;151(3):333-7.
245. Wong SF, Chan FY, Oats JJN, McIntyre DH. Fetal Growth Spurt and Pregestational Diabetic Pregnancy. Diabetes Care. 2002;25(10):1681. DOI: 10.2337/diacare.25.10.1681.
246. Pedersen NG, Wøjdemann KR, Scheike T, Tabor A. Fetal growth between the first and second trimesters and the risk of adverse pregnancy outcome. Ultrasound Obstet Gynecol. 2008 Aug;32(2):147-54. DOI: 10.1002/uog.6109.
247. Chen L, Wu J-J, Chen X-H, Cao L, Wu Y, Zhu L-J, et al. Measurement of Fetal Abdominal and Subscapular Subcutaneous Tissue Thickness during Pregnancy to Predict Macrosomia: A Pilot Study. PLOS ONE. 2014;9(3):e93077. DOI: 10.1371/journal.pone.0093077.
248. Khalifa EA, Hassanein SA, Eid HH. Ultrasound measurement of fetal abdominal subcutaneous tissue thickness as a predictor of large versus small fetuses for gestational age. Egyptian Journal of Radiology and Nuclear Medicine. 2019 2019/12/12;50(1):80. DOI: 10.1186/s43055-019-0088-6.
249. Bhat RG, Nathan A, R A, Vasudeva A, Adiga P, Bhat PV, et al. Correlation of fetal abdominal subcutaneous tissue thickness by ultrasound to predict birth weight. J Clin Diagn Res. 2014 Apr;8(4):Oc09-11. DOI: 10.7860/jcdr/2014/6498.4214.
250. Köşüş N, Köşüş A, Turhan N. Relation between abdominal subcutaneous fat tissue thickness and inflammatory markers during pregnancy. Arch Med Sci. 2014 Aug 29;10(4):739-45. DOI: 10.5114/aoms.2014.44865.
251. O'Brien CM, Louise J, Deussen A, Dodd JM. In overweight and obese women, fetal ultrasound biometry accurately predicts newborn measures. Australian and New Zealand Journal of Obstetrics and Gynaecology. [https://doi.org/10.1111/ajo.13025]. 2020 2020/02/01;60(1):101-7. DOI: https://doi.org/10.1111/ajo.13025.
252. Sparano S, Ahrens W, De Henauw S, Marild S, Molnar D, Moreno LA, et al. Being macrosomic at birth is an independent predictor of overweight in children: results from the IDEFICS study. Matern Child Health J. 2013 Oct;17(8):1373-81. DOI: 10.1007/s10995-012-1136-2.
253. Liu T, Mueller N, Benjamin-Neelon S. Identifying Mediators Underlying the Intergenerational Cycle of Obesity: A Causal Mediation Analysis. Current Developments in Nutrition. 2020;4(Supplement_2):1655-. DOI: 10.1093/cdn/nzaa063_053.
254. Walsh JM, McGowan CA, Mahony R, Foley ME, McAuliffe FM. Low glycaemic index diet in pregnancy to prevent macrosomia (ROLO study): randomised control trial. BMJ : British Medical Journal. 2012;345:e5605. DOI: 10.1136/bmj.e5605.

255. Hadlock FP, Harrist RB, Martinez-Poyer J. In utero analysis of fetal growth: a sonographic weight standard. Radiology. 1991 Oct;181(1):129-33. DOI: 10.1148/radiology.181.1.1887021.
256. Modi N. Time to bring fetal growth assessment into the 21st century. BJOG: An International Journal of Obstetrics & Gynaecology. [https://doi.org/10.1111/1471-0528.13974]. 2016 2016/11/01;123(12):1989-. DOI: https://doi.org/10.1111/1471-0528.13974.
257. Papageorghiou AT, Ohuma EO, Altman DG, Todros T, Cheikh Ismail L, Lambert A, et al. International standards for fetal growth based on serial ultrasound measurements: the Fetal Growth Longitudinal Study of the INTERGROWTH-21st Project. Lancet (London, England). 2014 Sep 6;384(9946):869-79. DOI: 10.1016/s0140-6736(14)61490-2.
258. Kiserud T, Piaggio G, Carroli G, Widmer M, Carvalho J, Neerup Jensen L, et al. The World Health Organization Fetal Growth Charts: A Multinational Longitudinal Study of Ultrasound Biometric Measurements and Estimated Fetal Weight. PLoS Med. 2017 Jan;14(1):e1002220. DOI: 10.1371/journal.pmed.1002220.
259. Stirnemann J, Villar J, Salomon LJ, Ohuma E, Ruyan P, Altman DG, et al. International estimated fetal weight standards of the INTERGROWTH-21(st) Project. Ultrasound Obstet Gynecol. 2017 Apr;49(4):478-86. DOI: 10.1002/uog.17347.
260. Jeffries K, Shub A, Walker SP, Hiscock R, Permezel M. Reducing excessive weight gain in pregnancy: a randomised controlled trial. Med J Aust. 2009 Oct 19;191(8):429-33. DOI: 10.5694/j.1326-5377.2009.tb02877.x.
261. Althuizen E, van der Wijden CL, van Mechelen W, Seidell JC, van Poppel MN. The effect of a counselling intervention on weight changes during and after pregnancy: a randomised trial. Bjog. 2013 Jan;120(1):92-9. DOI: 10.1111/1471-0528.12014.
262. Hui A, Back L, Ludwig S, Gardiner P, Sevenhuysen G, Dean H, et al. Lifestyle intervention on diet and exercise reduced excessive gestational weight gain in pregnant women under a randomised controlled trial. Bjog. 2012 Jan;119(1):70-7. DOI: 10.1111/j.1471-0528.2011.03184.x.
263. Luoto R, Kinnunen TI, Aittasalo M, Kolu P, Raitanen J, Ojala K, et al. Primary prevention of gestational diabetes mellitus and large-for-gestational-age newborns by lifestyle counseling: a cluster-randomized controlled trial. PLoS Med. 2011 May;8(5):e1001036. DOI: 10.1371/journal.pmed.1001036.
264. Phelan S, Phipps MG, Abrams B, Darroch F, Schaffner A, Wing RR. Randomized trial of a behavioral intervention to prevent excessive gestational weight gain: the Fit for Delivery Study. Am J Clin Nutr. 2011 Apr;93(4):772-9. DOI: 10.3945/ajcn.110.005306.
265. Poprzeczny AJ, Louise J, Deussen AR, Dodd JM. Effect of metformin in addition to an antenatal diet and lifestyle intervention on fetal growth and adiposity: the GRoW randomised trial. BMC endocrine disorders. 2020 Sep 14;20(1):139. DOI: 10.1186/s12902-020-00618-0.
266. Dodd JM, Cramp C, Sui Z, Yelland LN, Deussen AR, Grivell RM, et al. The effects of antenatal dietary and lifestyle advice for women who are overweight or obese on maternal diet and physical activity: the LIMIT

randomised trial. BMC medicine. 2014 Oct 13;12(1):161. DOI: 10.1186/s12916-014-0161-y.
267. Hjorth-Hansen A, Salvesen O, Engen Hanem LG, Eggebo T, Salvesen KA, Vanky E, et al. Fetal Growth and Birth Anthropometrics in Metformin-Exposed Offspring Born to Mothers With PCOS. J Clin Endocrinol Metab. 2018 Feb 1;103(2):740-7. DOI: 10.1210/jc.2017-01191.
268. Hui L. Australian charts for assessing fetal growth: a review. ASUM Ultrasound Bulletin. 2008 February, 2008:12 - 8.
269. Rowan JA, Rush EC, Obolonkin V, Battin M, Wouldes T, Hague WM. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition at 2 years of age. Diabetes Care. 2011 Oct;34(10):2279-84. DOI: 10.2337/dc11-0660.
270. Rowan JA, Rush EC, Plank LD, Lu J, Obolonkin V, Coat S, et al. Metformin in gestational diabetes: the offspring follow-up (MiG TOFU): body composition and metabolic outcomes at 7-9 years of age. BMJ Open Diabetes Res Care. 2018;6(1):e000456. DOI: 10.1136/bmjdrc-2017-000456.
271. Stephenson J, Heslehurst N, Hall J, Schoenaker D, Hutchinson J, Cade JE, et al. Before the beginning: nutrition and lifestyle in the preconception period and its importance for future health. Lancet (London, England). 2018 May 5;391(10132):1830-41. DOI: 10.1016/s0140-6736(18)30311-8.
272. Barker M, Dombrowski SU, Colbourn T, Fall CHD, Kriznik NM, Lawrence WT, et al. Intervention strategies to improve nutrition and health behaviours before conception. Lancet (London, England). 2018 May 5;391(10132):1853-64. DOI: 10.1016/s0140-6736(18)30313-1.
273. WEISSMANN-BRENNER A, SIMCHEN MJ, ZILBERBERG E, KALTER A, WEISZ B, ACHIRON R, et al. Maternal and neonatal outcomes of large for gestational age pregnancies. Acta obstetricia et gynecologica Scandinavica. 2012;91(7):844-9. DOI: https://doi.org/10.1111/j.1600-0412.2012.01412.x.
274. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ. 2005;331(7522):929. DOI: 10.1136/bmj.38586.411273.E0.
275. Monteiro PO, Victora CG. Rapid growth in infancy and childhood and obesity in later life--a systematic review. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2005 May;6(2):143-54. DOI: 10.1111/j.1467-789X.2005.00183.x.
276. Hediger ML, Overpeck MD, Maurer KR, Kuczmarski RJ, McGlynn A, Davis WW. Growth of infants and young children born small or large for gestational age: findings from the Third National Health and Nutrition Examination Survey. Archives of pediatrics & adolescent medicine. 1998 Dec;152(12):1225-31. DOI: 10.1001/archpedi.152.12.1225.
277. Kim SY, Sharma AJ, Sappenfield W, Wilson HG, Salihu HM. Association of maternal body mass index, excessive weight gain, and gestational diabetes mellitus with large-for-gestational-age births. Obstet Gynecol. 2014 Apr;123(4):737-44. DOI: 10.1097/aog.0000000000000177.
278. Ornoy A. Prenatal origin of obesity and their complications: Gestational diabetes, maternal overweight and the paradoxical effects of fetal growth

restriction and macrosomia. Reproductive toxicology (Elmsford, NY). 2011 Sep;32(2):205-12. DOI: 10.1016/j.reprotox.2011.05.002.
279. Scheil W JK, Scott J, Catcheside B, Sage L, Kennare R. Pregnancy Outcome in South Australia 2014. In: Pregnancy Outcome (Statistics) Unit SH, Government of South Australia, editor. Adelaide: SA Health; 2016.
280. Dodd JM, Turnbull DA, McPhee AJ, Wittert G, Crowther CA, Robinson JS. Limiting weight gain in overweight and obese women during pregnancy to improve health outcomes: the LIMIT randomised controlled trial. BMC pregnancy and childbirth. [Multicenter Study
Randomized Controlled Trial
Research Support, Non-U.S. Gov't]. 2011;11:79. DOI: 10.1186/1471-2393-11-79.
281. Owen P, Harrold AJ, Farrell T. Fetal size and growth velocity in the prediction of intrapartum caesarean section for fetal distress. British journal of obstetrics and gynaecology. 1997 Apr;104(4):445-9.