RANZCOG WOMEN'S HEALTH FOUNDATION RESEARCH SCHOLARSHIPS

NORMAN BEISCHER CLINICAL RESEARCH SCHOLARSHIP, 2018 - 2019

PROGRESS REPORT

Recipient: **Dr Amanda Poprzeczny**
Project Title: *'Maternal Overweight and Obesity and Gestational Diabetes: Effect on Fetal Growth and Adiposity'.*

Introduction/Background

The burden of overweight and obesity

Obesity, defined as a BMI >30.0kg/m2, represents a significant public health burden globally. Data from the most recent Pregnancy Outcome in South Australia report suggest that approximately 50% of women entering pregnancy in South Australia are overweight or obese (Schiel, 2013). The long term health risks of overweight and obesity include a greatly increased risk of cardiovascular disease, type 2 diabetes mellitus and several cancers, all of which result in reduced life expectancy and increased health-care expenditure.

Obesity and Fetal and Neonatal Growth

Infant birth weight is associated with many factors including gestational age at birth, infant birth order, smoking, pre-eclampsia and multiple pregnancy. Maternal pre-pregnancy weight and infant macrosoma during pregnancy are important factors in prediction of macrosomia (Dodd JM, 2011) (Cedergren, 2004).

Newborns of overweight and obese women have been shown to have significantly greater percentage of fat mass and total mass, and significantly less fat-free mass, when compared to those of normal weight women (Hull HR, 2008) (Sewell MF, 2006). This increase in fat mass may be an important mediator of later health, including childhood and adolescent obesity and metabolic syndrome. To date, however, ultrasound assessment of fetal growth, particularly two-dimensional measures of skeletal growth, have been poorly correlated with neonatal measures of body composition and body fat, regardless of how soon prior to delivery these ultrasound measures were obtained (Khoury F, 2009) (Lee W, 2009) (Moyer-Mileur LJ, 2009).

Ultrasound measures of fetal fat mass have been developed and normal ranges validated (Bernstein IM, 1991) (Gardeil F, 1999) (Higgins MF, 2008) (Hill LM, 1992) (Larciprete G, 2008). Fetal body composition measures most frequently examined to date include subscapular fat mass (SSFM), abdominal wall fat mass (fetal abdominal fat layer) (AFM), mid-thigh total mass (MTTM), mid-thigh lean mass (MTLM) and mid-thigh fat mass (MTFM) (Bernstein IM, 1991) (Gardeil F, 1999) (Higgins MF, 2008) (Hill LM, 1992) (Larciprete G, 2008). However, there is little published data regarding the effect of maternal BMI and gestational weight gain on fetal body composition.

Hure et al, in an Australian population, performed a prospective study investigating the effect of pre-pregnancy BMI and gestational weight gain on fetal growth parameters and fetal markers of adiposity (Hure AJ, 2011). While the methodology used is not directly comparable to that being used by our group, they showed that, independent of pre-pregnancy BMI, excessive gestational weight gain was associated with a larger fetus, predominantly as a result of an increase in lean abdominal mass, but not abdominal fat mass. A larger study performed by our group looked at fetal growth and markers of adiposity in overweight and obese women exposed to a diet and lifestyle intervention to limit gestational weight gain (Grivell RM, 2016), suggested a greater mid thigh fat mass and a slower rate of subscapular adipose tissue deposition among women exposed to the intervention. This suggests that the effects of maternal overweight and obesity on fetal fat mass can potentially be ameliorated by antenatal intervention.

Current fetal growth parameters and growth trajectories have been derived from healthy women, but have rarely considered the potential impact of maternal body mass index, using ultrasound assessment of femur length, abdominal circumference and in some circumstances, biparietal diameter and head circumference measurements. My proposal will involve performing an ultrasound examination for women participating in randomised trials of antenatal lifestyle interventions across the maternal BMI spectrum (The GRoW and OPTIMISE trials) at 28 and 36 weeks gestation to assess standard fetal growth parameters, and measures of adiposity. Ultrasonographic information related to fetal growth trajectories over time will allow the evaluation of the effect of the dietary and lifestyle intervention on in-utero fetal growth in a dynamic fashion.

Research from Professor Dodd's group has demonstrated that among overweight and obese pregnant women, provision of a dietary and lifestyle intervention is associated with a significant 18% relative risk reduction in the chance of an infant being born with weight above 4kg (Dodd BMJ 2014), and a 44% relative risk reduction in weight above 4.5kg (BMC Medicine 2014). Furthermore, the provision of the intervention generated modest but significant

improvements in maternal diet and physical activity (Dodd BMC Medicine 2014), which appear to persist to at least 6 months after birth (Dodd, unpublished data).

An important, but unanswered question arising from this work is how these observed changes among overweight and obese pregnant women compare with women of normal BMI, and specifically, whether an antenatal dietary maternal dietary change changes maternal diet among women of normal BMI, and the potential impact of such changes on fetal and neonatal growth.

I have been involved in performing fetal ultrasound at 28 and 36 weeks' gestation for women recruited to the GRoW and Optimise randomised trials, and collecting data on the ultrasound fetal biometry and adiposity measures. I have also been involved in data analysis and publication, and am currently working on writing up and publishing results of fetal biometry and adiposity measures gathered in the GRoW randomized trial.

Progress/Update

We have performed analysis of the mediating effects of gestational diabetes on fetal growth and adiposity in the LIMIT randomized trial cohort of women, and this has recently been published in the British Journal of Obstetrics and Gynaecology (attached). This work has shown that fetal growth and adiposity is not mediated by the diagnosis and treatment of gestational diabetes in this cohort of women who were overweight or obese in early pregnancy.

We have analysed data from the GRoW randomized trial, investigating the effect of a combined intervention of antenatal dietary and lifestyle advice and oral metformin on fetal biometry and adiposity measures among women who are overweight or obese in early pregnancy. I am currently in the process of writing up these results and submitting it for publication.

We have recently completed data collection for the Optimise randomized trial, investigating the effect of an antenatal dietary and lifestyle intervention on fetal growth and infant birthweight among women who have a normal BMI in early pregnancy. This cohort of women will represent the normal BMI comparator group, allowing us to perform analysis investigating the effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity. This data is in the process of being cleaned and analysed.

Conclusions

Thus far, my work has shown an important association between increased maternal BMI fetal growth, which is not mediated by treated gestational diabetes. We did not find an association between increased maternal BMI and fetal adiposity measures. We also did not find that this relationship was mediated by treated gestational diabetes. Future work will investigate whether a combined intervention of antenatal diet and lifestyle advice and oral metformin has an effect on fetal growth and adiposity, and will investigate the effect of maternal BMI, across the BMI spectrum, on fetal growth and adiposity.

Publication and/or Presentation of Results

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 randomized trial. BJOG 2018 Nov;125(12):1558-1566

Budget

My budget remains the same as that proposed in my scholarship application, providing salary supplementation and statistical support – 70% of the scholarship funding I have received has gone towards salary supplementation for myself, as I am working clinically part-time. The remainder of the scholarship funding (30%) has contributed to statistical analysis and data entry.

DISCIPLINE OF OBSTETRICS & GYNAECOLOGY
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PROVIDER NUMBER 2114806A

Thursday October 18, 2018

RANZCOG Women's Health Foundation

To Whom It May Concern:

Re: Progress Report, Amanda Josephine Poprzeczny

This is to inform you that Amanda is making adequate progress towards completion of her Norman Beischer Research Scholarship project, which forms part of her PhD project, being completed through the University of Adelaide. I expect that she will complete her work within the expected timeframe.

Kind regards

Professor Jodie Dodd
Discipline of Obstetrics & Gynaecology
NHMRC Practitioner Fellow
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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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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.

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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. To view this mini commentary visit https://doi.org/10.1111/1471-0528.15303.

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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

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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).

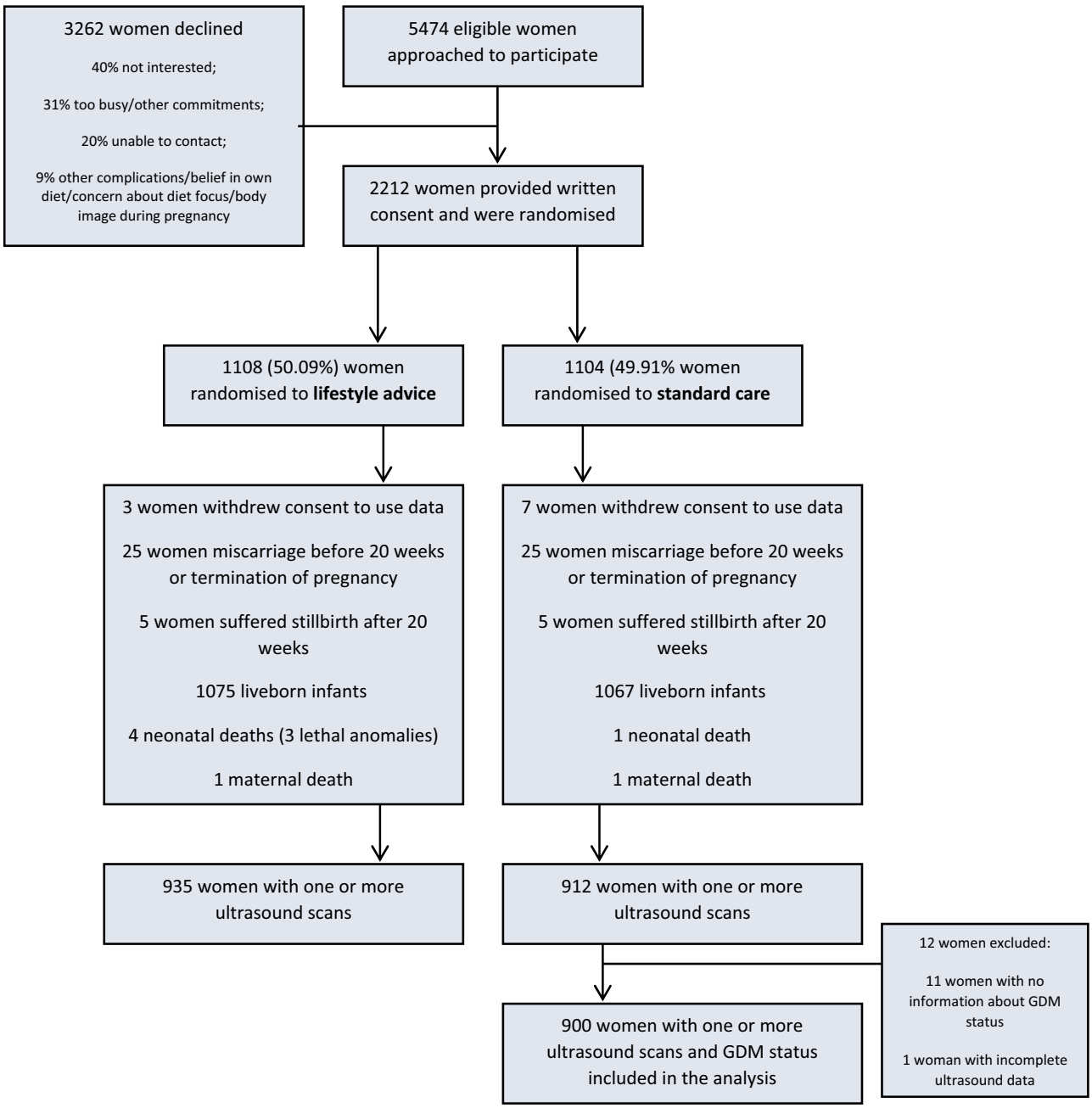

Figure 1. Flow of women eligible for inclusion in the analysis of ultrasound measurements and fetal growth and adiposity.

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).

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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. ■

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