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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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Abstract

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/m², and singleton gestation.

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Contribution to authorship

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.

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).

DISCLOSURES:

The authors have no conflicts of interest to declare. Completed disclosure of interest forms are available to view online as supporting information.

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Methods—Fetal ultrasound measures at 36 weeks' 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' 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, nor 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, nor 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

maternal obesity; gestational diabetes; fetal growth; fetal adiposity; fetal ultrasound

INTRODUCTION

Overweight and obesity, defined as BMI ≥ 25 kg/m² and BMI ≥ 30.0 kg/m² respectively, represent a significant public health burden globally (1). Across developed nations, 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 is associated with an increased risk of being born large for gestational age or having birth weight over 4kg (7, 8), perinatal morbidity and mortality (5, 7), and longer term risks of childhood obesity (9), (10).

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 birth weight over 4kg (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 3 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/m² (measured in early pregnancy), and who had a singleton pregnancy between 10⁺0 to 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 vs ≥ 30.0 kg/m²), and collaborating centre (16). Briefly, 5,474 eligible women were approached to participate, and 2,122 women were consented and randomised to the lifestyle advice group (1,018 women) or standard care (1,104 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 state-wide 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 state-wide clinical practice at the time, all women were encouraged to have a fasting 75g oral glucose tolerance test at 28 weeks' gestation (19). Using the clinical criteria in place at the time (22), gestational diabetes was diagnosed if the fasting blood glucose concentration was ≥ 5.5 mmol/L, or 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 two hours after the start of each meal (post-prandial)), with fasting blood glucose measurements targeted between 3.5–5.5 mmol/L, and post-prandial between 4.0–7.0 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 post-prandial 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' 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 into 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 were 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' gestation were mediated via diagnosed and treated GDM. For the purposes of analysis, BMI was divided into 2 categories (25.0–29.9 kg/m² and ≥ 30.0 kg/m²). 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 3 estimates for each outcome (see 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' 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, Texas, USA) (28, 29).

Patient 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.

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RESULTS

Participant 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' gestation. A total of 12 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 (1 woman) (Figure 1).

Baseline characteristics of participants are presented in Table 1. The mean maternal age at trial entry was 29.6 (standard deviation (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/m², 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 non-smokers (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 – 0.00] $p=0.183$). Similar findings were evident for both fetal abdominal 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 – 0.02] $p=0.532$; estimated fetal weight z-score indirect effect 0.00 [95% CI -0.01 – 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 cm² [95% CI -0.49 – 0.58 cm²] $p=0.877$ and total effect 0.07 cm² [95% CI -0.50 – 0.64 cm²] $p=0.802$; AFM direct effect 0.17 mm [95% CI -0.11 – 0.45 mm] $p=0.236$ and total effect 0.18 mm [95% CI -0.11 – 0.48 mm] $p=0.223$; SSFM direct effect 0.13 mm [95% CI -0.12 – 0.39 mm] $p=0.306$ and total effect 0.12 mm [95% CI -0.14 – 0.39 mm] $p=0.362$). There was no evidence of a mediated effect due to diagnosed and treated of GDM (MTFM indirect effect 0.03 cm² [95% CI -0.03 – 0.10 cm²] $p=0.352$; AFM indirect effect 0.01 mm [95% CI -0.02 – 0.05 mm] $p=0.402$; SSFM indirect effect -0.01 mm [95% CI -0.04 – 0.02 mm] $p=0.508$) (Table 3).

DISCUSSION

Main findings

Our findings demonstrate that in this population of overweight and obese women, while higher maternal BMI 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 utilised 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 state-wide 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 have 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 necessitates 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

While maternal overweight or obesity and GDM are recognised as risk factors for fetal overgrowth and birth weight 4kg (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 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 (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 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 (34). While 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 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 (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 intra-uterine milieu as observed in women who are overweight or obese, results 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’ 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 is 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 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 (39). Metabolic factors including circulating triglycerides (40, 41), leptin (42–44) and adiponectin (45, 46) 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. 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 of 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).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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

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
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: N(%)	
- No	798 (88.7)
- Yes	102 (11.3)
Parity: N(%)	
- 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)

Women were included in the analysis if they had an ultrasound at 28 or 36 weeks' gestation, or both.

* Number and %

** Mean and standard deviation

Socio-economic index as measured by SEIFA: www.abs.gov.au/websitedbs/censushome.nsf/home/seifa2011?opendocument&navpos=260

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, 0.28)	0.198
- Indirect Effect	−0.01 (−0.03, 0.01)	0.315
- Total Effect	0.10 (−0.08, 0.28)	0.271
HC z-score		
- Direct Effect	0.18 (0.05, 0.31)	0.005
- Indirect Effect	−0.01 (−0.02, 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, 0.02)	0.532
- Total Effect	0.26 (0.11, 0.42)	0.001
FL z-score		
- Direct Effect	0.09 (−0.06, 0.24)	0.256
- Indirect Effect	0.00 (−0.01, 0.02)	0.832
- Total Effect	0.09 (−0.06, 0.24)	0.244
EFW z-score		
- Direct Effect	0.22 (0.08, 0.35)	0.002
- Indirect Effect	0.00 (−0.01, 0.02)	0.901
- Total Effect	0.22 (0.08, 0.35)	0.002

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

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, 0.58)	0.877
- Indirect Effect	0.03 (−0.03, 0.10)	0.352
- Total Effect	0.07 (−0.50, 0.64)	0.802
AFM		
- Direct Effect	0.17 (−0.11, 0.45)	0.236
- Indirect Effect	0.01 (−0.02, 0.05)	0.402
- Total Effect	0.18 (−0.11, 0.48)	0.223
SSFM		
- Direct Effect	0.13 (−0.12, 0.39)	0.306
- Indirect Effect	−0.01 (−0.04, 0.02)	0.508
- Total Effect	0.12 (−0.14, 0.39)	0.362

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