Maternal visceral adiposity, a missing link in the prediction of birth weight centile

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Abstract

Context Maternal body mass index (BMI) is associated with increased birth weight but does not explain all the variance in fetal adiposity.

Objective To assess the contribution of maternal body fat distribution to offspring birth weight and adiposity.

Design Longitudinal study throughout gestation and at delivery.

Setting Women recruited at 12 weeks of gestation and followed up at 26 and 36 weeks. Cord blood was collected at delivery.

Patients Pregnant women (n=45) with BMI 18.0-46.3 kg/m^2 and healthy pregnancy outcome.

Methods Maternal first trimester abdominal subcutaneous and visceral adipose tissue thickness (SAT and VAT) was assessed by ultrasound.

Main outcome measures Maternal body fat distribution, maternal and cord plasma glucose and lipid concentrations, placental weight, birth weight and fetal adiposity assessed by cord blood leptin.

Results VAT was the only anthropometric measure independently associated with birth weight centile (r^2 adjusted 15.8%, *P*=0.002). BMI was associated with trimester 2 and trimester 1 – 3 area under the curve (AUC) glucose and insulin resistance (HOMA). SAT alone predicted trimester 2 lipoprotein lipase (LPL) mass (a marker of adipocyte insulin sensitivity) (11.3%, *P*=0.017). VAT was associated with fetal triglyceride (9.3%, *P*=0.047). Placental weight was the only independent predictor of fetal adiposity (48%, *P*<0.001). Maternal trimester 2 and AUC LPL were inversely associated with fetal adiposity (r=-0.69, *P*=0.001 and r=-0.58, *P*=0.006 respectively).

Conclusions Maternal VAT provides additional information to BMI for prediction of birth weight. VAT may be a marker of reduced SAT expansion and increased availability of maternal fatty acids for placental transport.

Precis

In pregnant women with a healthy pregnancy outcome, first trimester visceral adipose tissue predicted birthweight centile possibly due to increased delivery of fatty acids to the placenta.

Introduction

Maternal obesity occurs in around 20% of the UK antenatal population (1) and is associated with an increased risk of adverse pregnancy outcome (2) including the metabolic diseases of pregnancy, gestational diabetes mellitus (GDM) and pre-eclampsia. Maternal obesity is also associated with offspring obesity, both at birth and in later life (3). Studies show that maternal pre-pregnancy total body fat predicts birth weight, though it is unclear to what extent this is explained by fetal somatic growth or fetal accumulation of fat (4). There is increasing concern about the impact of maternal BMI on the long term metabolic health of the fetus (5).

Maternal obesity is associated with maternal insulin resistance (6) and metabolic dysfunction (7,8). GDM is defined by maternal hyperglycaemia and the associated fetal macrosomia may be explained by increased placental transport of glucose leading to increased fetal insulin secretion and hence increased growth, as described by Pedersen (9). This observation has now been extended to glucose tolerant mothers, as in the Hyperglycaemia and Adverse Pregnancy Outcome (HAPO) trial, which highlighted the importance of maternal plasma glucose concentrations for increased birth weight and adiposity in the offspring (5). However, plasma glucose concentration did not explain all the variance in offspring adiposity, with residual contributions coming from maternal pre-pregnancy BMI and gestational weight gain (10-12). Furthermore, increased birth weight is observed even in GDM pregnancies in which plasma glucose is well-controlled (10,13,14).

There is evidence to suggest that high maternal fat mass and insulin resistance may expose the fetus to fuels other than glucose that could contribute to higher birth weight (13,15-17). Maternal hypertriglyceridaemia is a key element of maternal obesity and insulin resistance (7,8). Whole body lipolysis is increased in the third trimester of pregnancy (18) and is associated with maternal fat mass and estimated fetal weight (19). Maternal plasma triglyceride and free fatty acid also correlate with birth weight and measures of neonatal adiposity in GDM and in women screened for GDM with normal glucose tolerance (20-22). However, it is not clear if these relationships exist in healthy non-obese pregnancy, to what extent they are independent of maternal body weight, and whether measures of maternal body fat distribution may be superior predictors of fetal birth weight and/or adiposity (23).

Obesity, as defined by BMI in excess of 30, is a universally accepted measure of body fatness, but BMI conveys no information about the quantity, quality, location or metabolic function of discrete fat depots. BMI is a relatively weak proxy for discriminating metabolic dysfunction and cardio-metabolic risk in comparison to central obesity, especially when the latter is distinguished by high intra-abdominal visceral adipose tissue (VAT)(24). BMI is unable to distinguish between individuals (pregnant or non-pregnant) who store fat as relatively benign subcutaneous adipose tissue (SAT) or as VAT, which is intimately associated with insulin resistance, hyperglycaemia, hypertriglyceridaemia, metabolic dysfunction and pathology (25-27). None of the above studies that related maternal adiposity to offspring birthweight and adiposity assessed body fat distribution.

In studies of pregnancy that did assess body fat distribution, pre-peritoneal and visceral fat were shown to increase during gestation, while SAT declined (28-32). Measures of VAT strongly predict metabolic complications of pregnancy, GDM (33,34) and pre-eclampsia (35,36), but it is not clear to what extent VAT is directly related to insulin resistance and increased plasma lipids and glucose in pregnancy (33,37-42). While VAT, measured by ultrasound between 12 and 20 weeks of gestation, is associated with fetal growth in overweight and obese women in the first trimester (43), with fetal growth and adiposity in the second trimester (44), and birthweight (45), the impact of abdominal SAT thickness on these measures has been largely overlooked. SAT thickness is correlated with first trimester fetal growth, but less so than VAT (43). SAT has also been found to be predictive (46-48) and non-predictive of GDM (49), and is also less strongly associated with metabolic risk factors in pregnancy than VAT (37).

The aim of this study was to assess the contribution of maternal body fat distribution to offspring birth weight and maternal and fetal insulin resistance in order to advance our understanding of the consequences of maternal obesity. Maternal first trimester measures of adiposity including BMI, VAT, abdominal SAT and hip circumference (a biomarker of lower body SAT) were assessed as predictors of birth weight, adiposity and metabolic dysfunction in neonates of healthy pregnant women. We also determined if any relationship could be explained by the influence of these fat depots on maternal glucose and lipid metabolism or placental weight.

Methods

Longitudinal Study of Pregnancy

Sixty women registered for obstetric care at the Princess Royal Maternity Unit, Glasgow, who were healthy and normotensive with no significant past medical history were recruited and followed prospectively throughout pregnancy. This study was initially designed to assess the impact of maternal obesity on microvascular function and powered for that outcome (50). Seven women were excluded from the final analysis; three delivered pre-term, two were excluded because of missing birth weight data, one had a miscarriage, and one had a baby with diGeorge syndrome. All remaining women had no pregnancy-related complications. Baseline data and complete longitudinal data were available for 53 and 45 women, respectively. The study was performed according to the Declaration of Helsinki, approval was granted by the Research Ethics Committee of North Glasgow University NHS Trust, and each subject gave written informed consent. The women attended after an overnight fast (>10 hours). Blood samples were collected at a mean of 12.4 (range 8–14) [T1], 26.1 (24–28) [T2] and 35.5 (33–38) [T3] weeks of gestation. The characteristics of the patients were recorded at the first antenatal hospital appointment and delivery details from patient notes. Customised birth weight centiles were calculated using the Gestation Network Centile Calculator 5.4 (http://www.gestation.net/birthweight_centiles/centile_online.htm). Deprivation category (DEPCAT score), a measure of socioeconomic status, was assigned using the Scottish Area Deprivation Index for Scottish postcode sectors, 1998 (51). Placental

tissue and fetal cord blood was collected at delivery from a subgroup of these pregnancies (n=23, 42%).

Baseline anthropometric and fat thickness measurements

At the first antenatal appointment (mean 12.4 weeks of gestation), patient height, weight, waist circumference and hip circumference were measured. Waist circumference was measured at the level of the umbilicus. Hip circumference was measured at the widest point over the buttocks. Waist and hip circumference were measured in duplicate to the nearest 0.5 cm. If the difference between the two measurements was greater than 2 cm, a third measurement was taken and the mean of the two closest measurements was calculated. All measurements were taken by the same examiner. Body mass index (BMI) was calculated as booking weight (kg), divided by height (m) squared. Baseline upper body abdominal SAT and upper body VAT thickness was assessed by ultrasound (52). Measurements were taken 2cm below the xiphisternum and the abdominal probe was placed on the skin with minimal pressure. Abdominal SAT was measured as the thickness between the inferior border of the dermal layer and the rectus abdominus sheath at the level of the umbilicus. Visceral fat was taken as the vertical measurement between the rectus sheath and the aorta at the umbilicus. Three consecutive measurements in millimetres were taken and an average reading was calculated. All measures were made by the same operator (F.S.) on the same machine.

Blood parameters

Glucose assays (53) were performed by Clinical Biochemistry, Glasgow Royal Infirmary and plasma total cholesterol, HDL cholesterol and total triglyceride concentrations were determined as described previously (7). Insulin (Mercodia) and leptin (R & D Systems) analyses were performed by ELISA according to the manufacturer's instructions. LPL mass was determined by ELISA using bovine LPL as standard (54,55). HOMA was calculated as [fasting insulin (mU/L) x fasting glucose (mmol/L)]/22.5]. Erythrocyte membrane phospholipid fatty acid composition was measured as previously described (56), and the ratio of 16:0/18:2 n-6 was used as an index of *de novo* lipogenesis (57,58).

Statistical analysis

Continuous variables are represented as means (standard deviation), categorical variables as number and percentage. Total area under the time (T1 to T3 weeks' gestation) x concentration curve (AUC) were calculated using the trapezium method (59). Normality testing was carried out using the Ryan-Joiner test and data was log or square root transformed to achieve a normal distribution as necessary. Associations between variables were examined by Pearson's correlation analysis. Two measures of gestational fuel exposure were used. Firstly, the total area under the trimester 1 to trimester 3 maternal glucose, and triglyceride concentration and HOMA curves were calculated to assess total gestational exposure. Secondly, because the univariate associations between maternal anthropometrics and maternal plasma triglyceride, glucose and HOMA were the strongest and notably distinct in trimester 2 (Supplemental Figure 1), trimester 2 data were selected for further study. Stepwise regression analysis, a method of fitting regression models in which the

choice of predictive variables and simultaneous removal of unimportant variables is carried out by an automatic procedure, was used to test associations between maternal anthropometrics and maternal or fetal blood glucose and lipids and the influence of confounding variables such as placental weight, using *P*-to-enter and *P*-to-stay $P<0.15$. All statistical analysis was carried out in Minitab Vs18.

Results

Maternal characteristics

Demographic data for all women with first trimester measurements are shown in Table 1. Women were on average 28 years of age, had a mean body mass index (BMI) of 28 kg/m^2; just over one third were currently smoking during their pregnancy and just less than one half were in their first pregnancy. More than half the women were classed as having deprived social status. The women had normal antenatal appointment blood pressure, had no pregnancy-related complications and all delivered healthy babies at term. The demographic characteristics of the subgroup, where repeated longitudinal measures were available, were similar to the total group (Table 1).

Relationship between maternal anthropometrics and offspring birth weight centile and placental weight

Maternal BMI is a recognised predictor of offspring birthweight. To test whether other, more specific, measures of maternal adiposity might be better predictors of offspring birth weight, univariate correlation between maternal anthropometric measures and birth weight centile, birth weight and placental weight were first assessed. Birth weight centile was associated with maternal BMI (r=0.41, *P*=0.002), waist circumference (r=0.42, *P*=0.003), hip circumference (r=0.32, *P*=0.021), SAT thickness (r=0.34, *P*=0.012), VAT thickness (r=0.41, *P*=0.003) and SAT plus VAT thickness (r=0.39, *P*=0.004), but not waist-hip ratio (r= 0.16, *P*=0.25) or VAT/SAT ratio (r=0.01, *P*=0.97). No maternal anthropometric measures were associated with birth

weight alone, and only maternal BMI was weakly associated with placental weight (r=0.27, *P*=0.047).

To examine possible multivariable associations between maternal anthropometrics and birth weight centile, the former was entered into a stepwise regression model (*P* to enter and *P* to stay 0.15). VAT was the only anthropometric measure significantly associated with birth weight centile (r^2 adjusted 15.8%, *P*=0.002, Figure 1). A one mm increase in VAT thickness resulted in a 2.26 centile increase in birthweight centile. A VAT thickness of up to 10mm was associated with the 43rd (unadjusted) and 38th (after adjustment for BMI, waist circumference, hip circumference, SAT thickness and SAT plus VAT thickness) birth weight centile. Above 10mm VAT, birth weight centiles were between the 70th and 80th centile. While inclusion of placental weight in the regression model attenuated the relationship between VAT and birth weight centile, it remained significant [r^2 adjusted 11.8%, *P*=0.006; birth weight centile = -12.3 + 1.891 VAT (mm) + 0.0630 placental weight (g)] suggesting an independent association between VAT thickness and birth weight centile. Placental weight was significantly associated with birth weight centile in this model (r^2 adjusted 12.1%, *P*=0.005), suggesting that placental weight also has an independent contribution to birth weight equivalent to that of VAT thickness.

Relationships between maternal anthropometrics and fetal cord plasma glucose and lipids

In a univariate analysis maternal VAT thickness was significantly correlated (*P*<0.05) with fetal cord plasma total cholesterol, triglyceride, non-esterified fatty acids and negatively with a marker of insulin resistance (HOMA), but there was no relationship

252 with fetal cord plasma HDL cholesterol, glucose or insulin (Table 2). Maternal SAT
253 thickness correlated significantly with cord plasma total cholesterol. There was no
254 relationship between maternal BMI or hip circumference and cord plasma glucose or
255 lipids. On multivariate regression analysis, VAT showed significant independent
256 associations with cord plasma triglyceride, non-esterified fatty acids and cholesterol,
257 while BMI showed significant negative independent associations with cord plasma
258 insulin and HOMA (Table 2). After inclusion of mode of delivery in the model, the
259 associations between maternal BMI and cord plasma insulin and HOMA and
260 between VAT and cord plasma triglyceride persisted and now hip circumference was
261 positively associated with cord plasma cholesterol (Table 2). The inclusion of
262 placental weight in the regression model had no impact on the associations between
263 BMI and cord plasma HOMA, VAT thickness and cord plasma triglyceride or between
264 hip circumference and cord plasma cholesterol maternal BMI and HOMA. The
265 inclusion of placental weight strengthened the negative association between BMI
266 and cord blood insulin (r^2 adjusted 31%, P=0.009).
267
268 *Relationship between maternal anthropometrics and maternal plasma glucose and*
269 *lipids*
270 Maternal BMI was correlated significantly (P<0.05) with maternal trimester 2 and
271 AUC glucose and trimester 2 and AUC HOMA (Table 3). SAT thickness was correlated
272 significantly with maternal trimester 2 HOMA only. VAT thickness was correlated
273 with maternal trimester 2 glucose and HOMA. Hip circumference was correlated
274 with maternal AUC glucose and trimester 2 and AUC HOMA. On stepwise regression,

only maternal BMI remained significantly associated with both maternal trimester 2 and AUC glucose and HOMA (Table 3).

Relationships between maternal plasma glucose and lipid exposure and fetal cord plasma glucose and lipids, fetal adiposity (cord plasma leptin) and birth weight centile

None of the markers of maternal glucose or lipid gestational exposure were associated with any measure of cord plasma glucose or lipid metabolism or fetal adiposity before or after accounting for mode of delivery other than maternal AUC triglyceride (r^2 = 20%, *P*=0.020, coefficient = -0.007) and maternal AUC HOMA (r^2=15%, *P*=0.042, coefficient = 0.255) which were associated with cord plasma HDL after accounting for mode of delivery. Maternal trimester 2 HOMA (r=0.33, *P*=0.028) and AUC glucose (r=0.36, *P*=0.016) were univariately associated with birthweight centile. T2 HOMA (*P*=0.046) and placental weight (*P*<0.001) remained as predictors of birthweight centile in a minimal model that included these two variables and AUC glucose (r^2 adjusted =31.7%).

Maternal anthropometric measures and fetal adiposity

Fetal adiposity was not associated with maternal BMI, SAT or VAT thickness or hip circumference in multivariate analysis. However, there was a significant positive association between placental weight and cord plasma leptin (r^2=57%, *P*<0.001). A multivariable model including maternal anthropometrics, placental weight and mode of delivery showed that only placental weight was independently associated with fetal adiposity (r^2 adjusted 48%, *P*<0.001). There was no association between cord

plasma triglyceride or non-esterified fatty acids and fetal adiposity even after adjusting for mode of delivery. The 16:0/18:2 n-6 ratio of fetal erythrocyte fatty acids was used as an index of *de novo* lipogenesis. This index was unrelated to fetal adiposity (r=-0.13, *P*=0.57), but inversely associated with fetal cord plasma triglyceride (r=-0.44, *P*=0.044) (Supplemental Figure 2), an effect lost after accounting for mode of delivery.

Maternal lipoprotein lipase (LPL) mass, maternal and fetal plasma glucose and lipids, fetal adiposity and birth weight centile

Low LPL mass is a marker of severity of metabolic syndrome and low plasma levels reflect reduced LPL synthesis by adipocytes in the insulin resistant state. Maternal trimester 2 LPL mass was negatively correlated with maternal BMI (r=-0.36, *P*=0.017) and SAT (r=-0.36, *P*=0.018) while AUC LPL mass correlated with SAT (r=-0.30, *P*=0.048). In a multivariate regression model including all maternal anthropometrics, maternal SAT alone predicted trimester 2 LPL mass (r^2 adjusted 11.3%, *P*=0.017). Maternal trimester 2 or AUC LPL mass was not correlated with cord plasma glucose or lipid levels. Maternal trimester 2 LPL was negatively correlated with maternal trimester 2 glucose (r=-0.30, *P*=0.049), AUC glucose (r=-0.36, *P*=0.019) and trimester 2 HOMA (-0.30, *P*=0.048). In particular, maternal trimester 2 and AUC LPL mass were strongly correlated with maternal trimester 2 triglycerides (r=-0.52, *P*=0.001 and r=-0.55, *P*<0.001 respectively) and AUC triglycerides (r=-0.41, *P*=0.007 and r=-0.41, *P*=0.006 respectively). Maternal trimester 2 and AUC LPL were not associated with birth weight centile but both were strongly associated with fetal leptin (r=-0.69,

322 *P*=0.001 and r=-0.58, *P*=0.006 respectively) (Figure 2) and these associations were

323 independent of mode of delivery.

Discussion

Maternal first trimester VAT thickness on ultrasound, but not first trimester BMI, abdominal SAT or hip circumference, was independently associated with birth weight centile. This observation is in agreement with a similar study in adolescent mothers, although the previous study lacked SAT assessment (45). The strength of associations in the current study were of greater magnitude than that previously reported, possibly due to our older, more obese population. Maternal VAT was also associated with fetal cord plasma triglyceride although this variable was not related to birth weight centile or fetal adiposity. Maternal VAT was not associated with maternal plasma lipids, as might be expected from data in non-pregnant women, in which an oversupply of fatty acids in the portal circulation to the liver can drive an increased synthesis and secretion of VLDL (60). This lack of relationship between maternal VAT and plasma triglyceride suggests that VAT-associated hypertriglyceridaemia may be superseded by maternal metabolic adaptation to pregnancy.

We have previously shown in *ex vivo* adipocyte lipolysis experiments, that SAT adipocytes have higher lipolysis rates than VAT adipocytes in pregnancy (61). Thus, in healthy pregnancy, SAT rather than VAT is the primary source of the maternal fatty acids released for maternal metabolism and placental transport, secondary to pregnancy hormone induced gestational insulin resistance (61). A slowing or reversal of maternal (subcutaneous) adipose tissue accumulation towards the end of the second trimester coincides with the accelerated phase of fetal adipose tissue accretion (14). In pregnancy, it is possible that VAT is a marker of ectopic fat

accumulation rather than a direct source of lipids for transport to the fetus. In non-pregnant individuals, fat accumulation in VAT and ectopically in other organs is secondary to dysregulated adipocyte expansion in subcutaneous fat (24,62,63). Fatty acids that overspill from the SAT compartment accumulate in VAT and are stored ectopically as intracellular lipid droplets in other tissues such as the liver and pancreas. However, in pregnancy, the overspill fatty acids from SAT could also be available for uptake and transport by the placenta, thus increasing lipid supply to the fetus (Figure 3).

Low maternal LPL mass is a measure of metabolic syndrome severity and probably reflects a reduced rate of LPL synthesis by insulin resistant SAT adipocytes (7,64-66). In the present study, LPL mass was inversely associated with maternal plasma triglyceride and fetal adiposity, supporting the idea of a failure of SAT adipocyte expansion and the development of adipocyte insulin resistance with a consequent overspill of fatty acids and transport to the fetus (Figure 3). Potential mechanisms for increased fetal adiposity include an increased lipid supply across the placenta or increased *de novo* lipogenesis from glucose supplied across the placenta. The inverse association between cord plasma triglyceride and an index of fetal *de novo* lipogenesis was not independent of mode of delivery, and in any case would suggest that the transported fatty acids may be utilised by the fetus in preference to the *de novo* synthesis of fatty acids. Failure of SAT adipocyte expansion has been proposed to underlie obesity-related pre-eclampsia (61) and gestational diabetes mellitus (67,68). In the healthy women under study here, the maximum VAT thickness measured was 27.3mm which may represent a propensity towards limited SAT

372 expansion rather than a pathological state, especially when it is considered that pre-
373 eclampsia and GDM are predicted by VAT thickness above 52mm (36) and 47.4mm
374 (33) respectively.
375
376 Maternal placental weight had an independent association with birth weight centile
377 in this cohort of women. Maternal body mass index was related to both placental
378 weight and maternal insulin resistance, and through these associations may be
379 indirectly linked to birth weight centile and fetal adiposity. Our data showed that
380 trimester 2 markers of glucose metabolism were most strongly related to maternal
381 anthropometrics. The middle trimester is the time of greatest acceleration in fetal
382 growth (69), when changes in maternal metabolism would be expected to have most
383 impact on birthweight centile. Maternal gestational insulin resistance directs more
384 glucose for transport to the fetus. A combination of higher placental weight,
385 increased surface area for transport of nutrients, and raised trimester 2 plasma
386 glucose may explain the link between BMI and fetal birth weight centile and
387 adiposity. Interestingly, maternal BMI was associated with reduced fetal cord blood
388 insulin and HOMA suggesting that fetuses of healthy high BMI mothers are more
389 insulin sensitive and hence efficient at storing fuel as adipose tissue in addition to
390 having more insulin-induced somatic growth. It is not clear whether fetal insulin
391 sensitivity is directly influenced by the mother or is a fetal response to the
392 availability of fuel.
393
394 The data presented here suggest an input from both glucose and lipids into birth
395 weight and fetal adiposity. While there is plentiful evidence to link maternal plasma

glucose levels with fetal growth, there has been less research into the role of plasma lipids. Lipid concentrations are equally regulated by insulin and affected by insulin resistance. It is notable that a stable isotope tracer study in healthy women at 34-36 weeks of gestation showed that both glucose production rate and lipolysis were independently correlated with estimated fetal size on ultrasound scan (19). Our data also suggest that glucose and lipid metabolism are intertwined and influenced by both maternal adiposity and body fat distribution.

This study had a number of strengths including its prospective design and the assessment of a number of maternal anthropometrics in parallel with measures of both maternal and fetal cord glucose and lipid metabolism. Limitations include a lack of kinetic assessment of maternal and fetal metabolites and the use of steady state concentrations to infer such fluxes. Our conclusions with respect to fetal adiposity are limited by cord blood samples being collected in less than half of the cohort, in which plasma leptin concentration was measured as a surrogate.

In summary, maternal body fat distribution in healthy pregnancy, as identified by VAT thickness, provides additional information to that of maternal BMI in the prediction of birth weight centile. VAT may be acting as a marker of reduced SAT expansion, leading to increased availability of plasma fatty acids for placental transport.

Data availability

The datasets generated during and analysed during the current study are not publically available but are available from the corresponding author on reasonable request.

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672 **Table 1. Maternal antenatal booking characteristics.** Values are mean and standard
673 deviation (SD) for continuous variables or number (%) for categorical variables. †n=1 missing
674 data.

	Baseline data n=53	Longitudinal data n=45
Booking visit		
Age (years)	28.3 (5.1)	28.6 (5.0)
Smokers (number (%))	19 (35.8)	16 (35.6)
Deprivation Index		
DEPCAT Score (number (%))		
Affluent (1-2)	5 (9.4)	5 (11.1)
Intermediate (3-5)	18 (34.0)	16 (35.6)
Deprived (6-7)	30 (56.6)	24 (53.3)
Primiparous	25 (47.2)	21 (46.7)
Systolic Blood Pressure (mmHg)	118 (13)	119 (11)
Diastolic Blood Pressure (mmHg)	68 (9)	69 (9)
BMI (kg/m^2)	28.4 (6.0)	28.1 (5.7)
BMI range (kg/m^2)	18.0 – 46.3	18.0 – 46.3
Waist circumference (cm)	89.5 (14.8)	89.5 (14.8)
Hip circumference (cm) †	107 (15)	108 (15)
Waist/Hip ratio	0.83 (0.08)	0.84 (0.08)
Visceral fat thickness (mm)	13.4 (5.6)	13.2 (5.5)
Subcutaneous fat thickness (mm)	25.1 (11.4)	24.5 (10.9)
Visceral/Subcutaneous fat thickness	0.58 (0.23)	0.58 (0.24)
Visceral plus subcutaneous fat thickness (mm)	38.4 (15.7)	37.7 (15.1)
At delivery		
Gestation at delivery (days)	279 (9)	281 (7)
Fetal sex, number (%) male	29 (55)	24 (53)
Placental weight (g)	763 (175)	785 (175)
Birth weight (g)	3606 (555)	3680 (560)
Birth Weight Centile	61 (31)	62 (31)

675

Table 2 Maternal BMI, VAT thickness, SAT thickness and hip circumference association with fetal cord plasma markers of glucose and lipid metabolism

Mean (standard deviation) cord plasma levels (n=23) of each parameter are shown below along with their univariate association (Pearson's correlation) with maternal BMI, VAT and SAT thickness and hip circumference. The relationship between maternal BMI, VAT and SAT thickness and fetal cord plasma metabolic measures was determined by entering BMI, SAT, VAT and hip circumference in a stepwise regression model *P* to enter and *P* to stay 0.15. Mode of delivery (MOD) [assisted (A), elective Caesarean section (L), emergency Caesarean section (M) and vaginal (V) delivery] was added to the models as a confounding variable. NEFA = non-esterified fatty acid. r^2 adjusted and *P* values are stated. *analysis carried out on log-transformed data. † *P*<0.05, ‡ no terms were at *P*<0.15 to be entered into the model.

Response	Cord plasma mean (SD)	Obesity measure	Univariate correlation	Coefficient	Contribution to variance, multivariable	*P*	Contribution to variance, multivariable including MOD	*P*
Glucose (mmol/L)	4.30 (1.07)	BMI	-0.17	-0.0655	VAT 6.9%	0.013	No model‡	-
		SAT	-0.07					
		VAT	-0.34					
		Hip	0.08					
Insulin* (mU/L)	6.56 (6.64)	BMI	-0.36	-0.0332	BMI 15.4%	0.040	BMI 15.4%	0.040
		SAT	-0.37					
		VAT	-0.37					
		Hip	-0.36					
HOMA-IR*	1.29 (1.21)	BMI	-0.38	-0.0596	BMI 20.5%	0.023	BMI 20.5%	0.023

Response	Cord plasma mean (SD)	Obesity measure	Univariate correlation	Coefficient	Contribution to variance, multivariable	*P*	Contribution to variance, multivariable including MOD	*P*
		SAT	-0.35					
		VAT	-0.45†					
		Hip	-0.28					
Triglyceride (mmol/L)*	0.72 (0.65)	BMI	0.16	0.01994	VAT 14.8%	0.043	VAT 9.3%	0.047
		SAT	0.30					
		VAT	0.42†					
		Hip	0.14					
NEFA* (mmol/L)	0.19 (0.12)	BMI	0.26	0.0236	VAT 17.5%	0.034	VAT 12.8%	0.075
		SAT	0.26					
		VAT	0.47†					
		Hip	0.28					
Total cholesterol* (mmol/L)	1.79 (0.65)	BMI	0.33	0.00938	VAT 14.0%	0.049	Hip 16.8%	0.002
		SAT	0.42†					
		VAT	0.43†					
		Hip	0.33					
HDL cholesterol (mmol/L)	0.74 (0.18)	BMI	0.12		No model‡		-	-
		SAT	0.02					
		VAT	-0.11					
		Hip	0.15					

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Table 3 Maternal BMI, abdominal visceral and subcutaneous adipose tissue and hip circumference associations with maternal markers of gestational fuel exposure Mean (standard deviation) maternal plasma trimester 2 levels or AUC trimester 1 to trimester 3 (n=45) are shown below along with their univariate association (Pearson's correlation) with maternal BMI, VAT and SAT thickness and hip circumference.

The relationship between maternal BMI, visceral adipose tissue (VAT) and upper body subcutaneous adipose tissue (SAT) thickness and maternal plasma metabolic measures was determined by entering BMI, SAT, VAT and hip circumference in a stepwise regression model *P* to enter and *P* to stay 0.15. r^2 adjusted and *P* values are stated. *analysis carried out on log-transformed data. † *P*<0.05, ‡ no terms were at *P*<0.15 to be entered into the model.

Response	Maternal plasma mean (SD)	Obesity measure	Univariate correlation	Coefficient	Contribution to variance, multivariable	*P*
Maternal T2 Glucose (mmol/L)*	5.41 (1.17)	BMI	0.38†	0.00591	BMI 12.5%	0.011
		SAT	0.20			
		VAT	0.32†			
		Hip	0.28			
Maternal T2 Triglyceride (mmol/L)*	2.41 (0.73)	BMI	0.20	0.00348	SAT 5.1%	0.078
		SAT	0.27			
		VAT	0.17			
		Hip	0.12			
Maternal T2 HOMA*	12.3 (10.8)	BMI	0.41†	0.0313	BMI 15.1%	0.005
		SAT	0.31†			
		VAT	0.37†			
		Hip	0.33†			
Maternal AUC glucose (mmol/l x weeks)*	120 (26)	BMI	0.39†	0.00563	BMI 15.3%	0.009
		SAT	0.17			
		VAT	0.27			
		Hip	0.34†			
Maternal AUC triglyceride (mmol/L x weeks)	50 (14)	BMI	0.18		No model‡	
		SAT	0.27			
		VAT	0.06			
		Hip	0.15			
Maternal AUC HOMA (HOMAx	240 (186)	BMI	0.35†	0.02074	BMI 10.0%	0.021
		SAT	0.25			
		VAT	0.24			

Response	Maternal plasma mean (SD)	Obesity measure	Univariate correlation	Coefficient	Contribution to variance, multivariable	*P*
weeks)*		Hip	0.31†			

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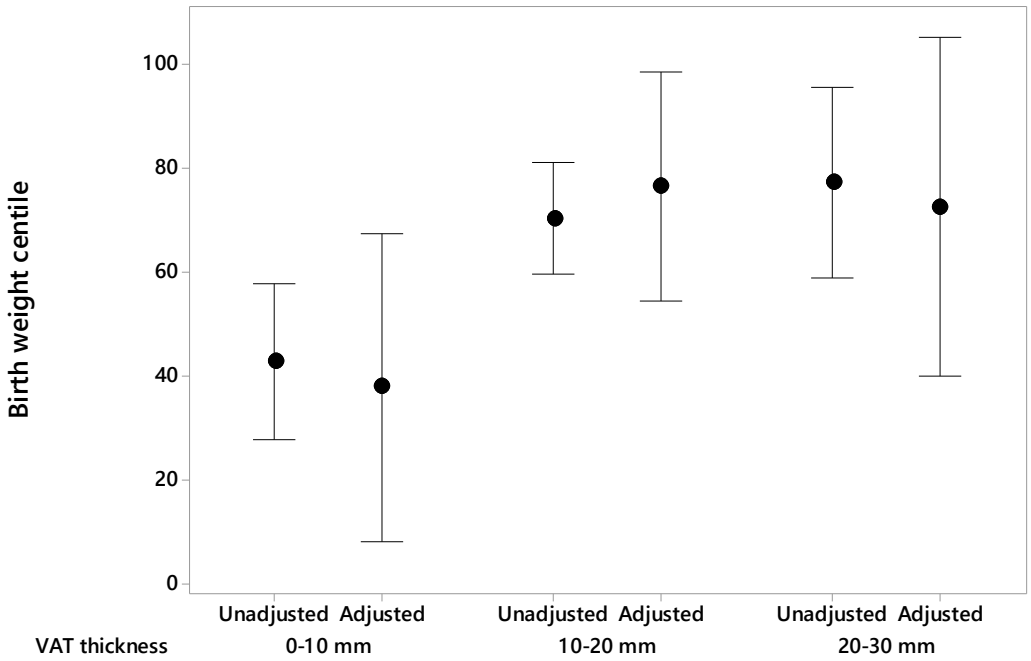
Legends for figures and tables

Figure 1. Offspring birth weight centile by VAT thickness: 0-10mm [n=19, mean (standard deviation) 7.5 (2.0) mm], 10-20mm [n=27, 15.0 (2.6) mm], 20-30mm [n=7, 22.9 (2.9) mm]. Means and 95% confidence interval for the mean are plotted for unadjusted data and data adjusted for other anthropometric measures in a stepwise regression (BMI, waist circumference, hip circumference, SAT thickness, SAT plus VAT thickness).

Figure 2. Association between fetal adiposity (cord plasma leptin) and maternal trimester 2 lipoprotein lipase mass. Univariate correlation (Pearson's) between maternal trimester 2 lipoprotein lipase mass and fetal adiposity r=-0.69 *P*=0 .001 (n=2 data missing).

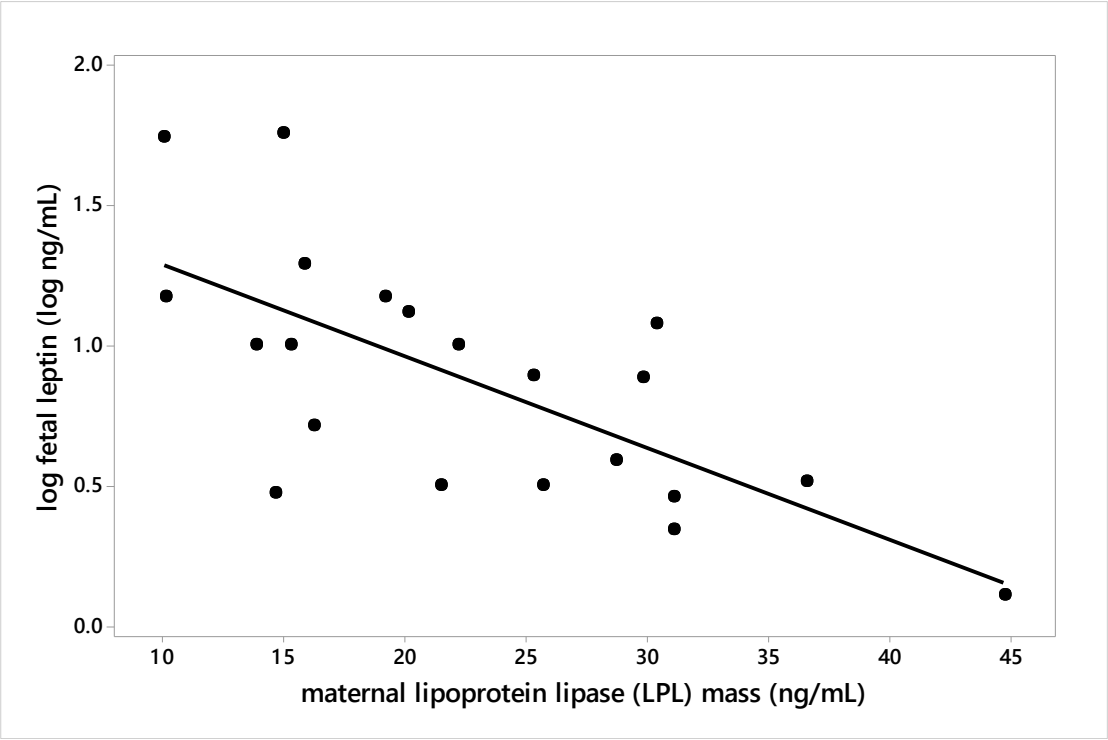
Figure 3. Proposed pathway for the contribution of maternal BMI, SAT and VAT to fetal birth weight and adiposity. In low BMI pregnant women subcutaneous adipose tissue (SAT) contains insulin sensitive (IS), hyperplasic adipocytes secreting large amounts of lipoprotein lipase (LPL) that are capable of expanding to store excess fatty acids (NEFA). In an insulin sensitive environment, this facilitates regulation of maternal glucose and triglyceride concentrations providing sufficient fuels for placental transport to support healthy fetal growth. In high BMI pregnant women, SAT contains insulin resistant (IR), hypertrophic adipocytes resulting from limited expansion of pre-adipocytes to form mature adipocytes. SAT has a reduced ability to store fatty acid which spill over and are directed to the liver increasing plasma triglyceride (TG) concentrations and are stored ectopically in visceral adipose tissue (VAT). The increasingly insulin resistance environment also raises plasma glucose levels thus increasing the supply of both fuels across a larger placenta resulting in a larger and fatter fetus.

723 **Figure 1**

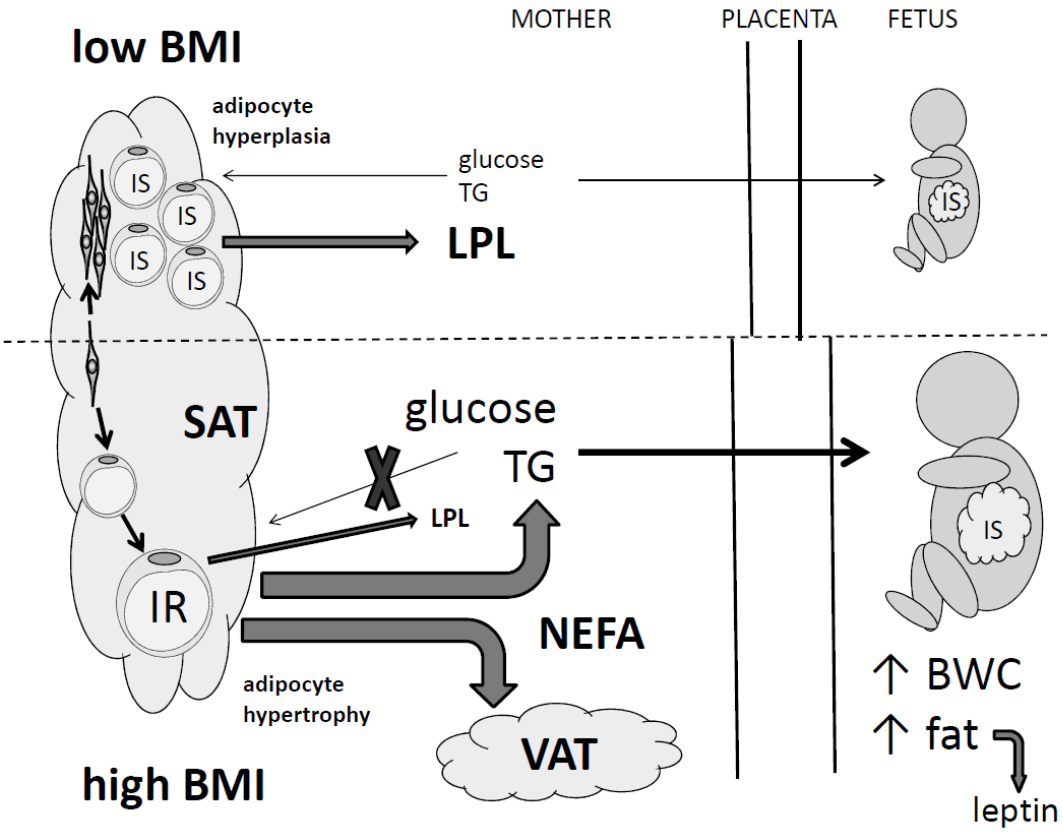


724

Figure 2.


2.0
1.5
1.0
0.5
0.0
log fetal leptin (log ng/mL)
10
15
20
25
30
35
40
45
maternal lipoprotein lipase (LPL) mass (ng/mL)

729 **Figure 3**



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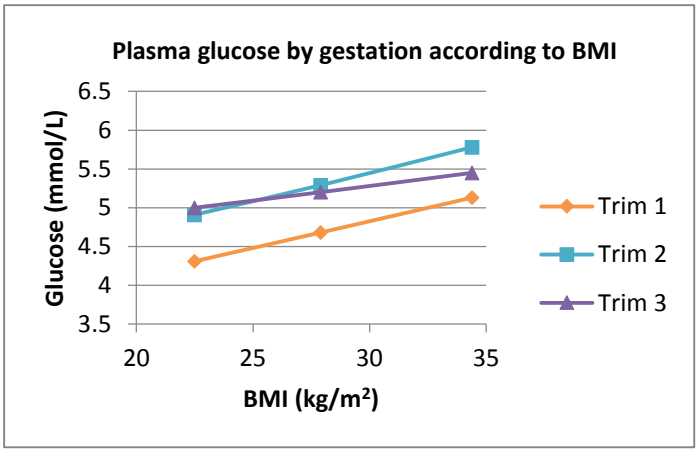
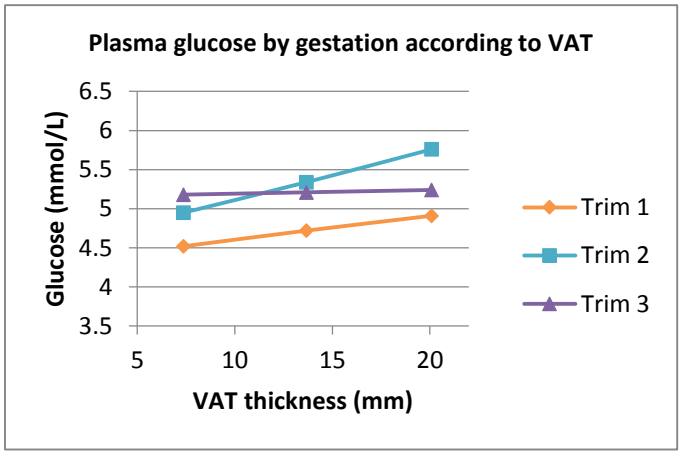
Supplemental Material

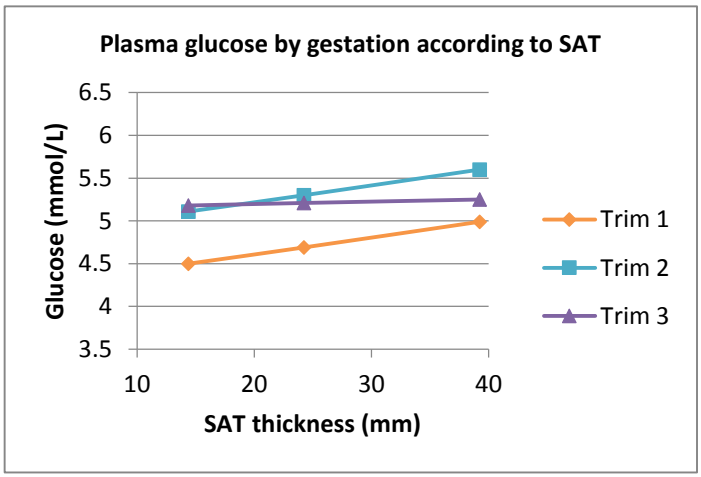
Supplemental Figures

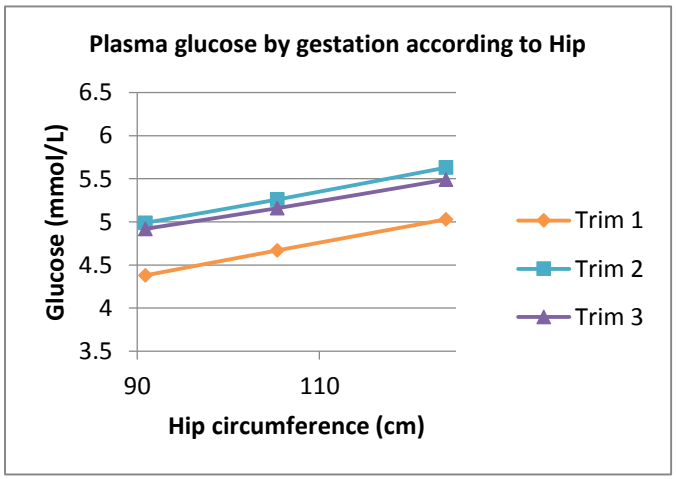
Supplemental Figure 1. Glucose, triglyceride and HOMA by trimester according to BMI, VAT thickness, SAT thickness and hip circumference. Univariate associations between maternal anthropometric measures and maternal plasma glucose (A) and triglyceride (B) concentration, and maternal HOMA (C) are shown.

A

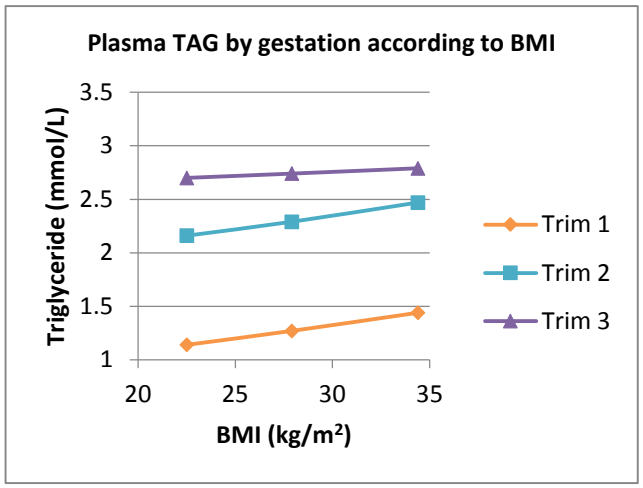


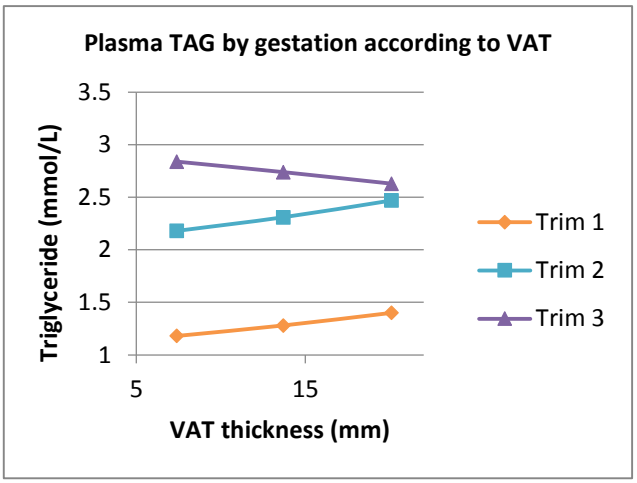


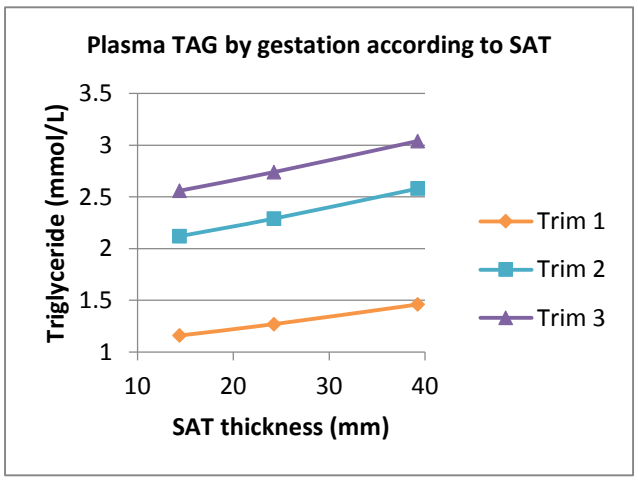


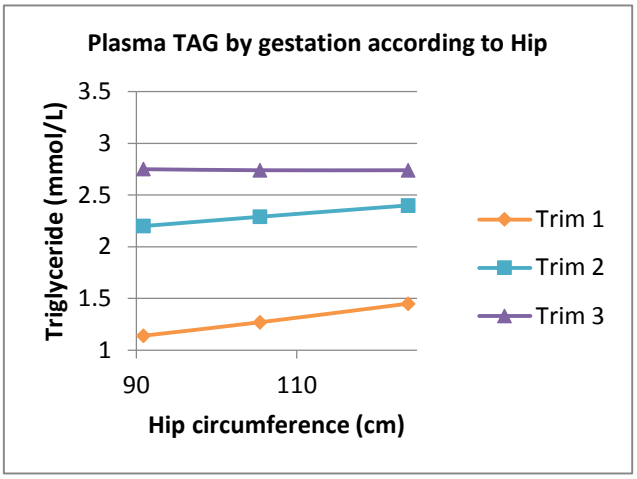


740 **B**

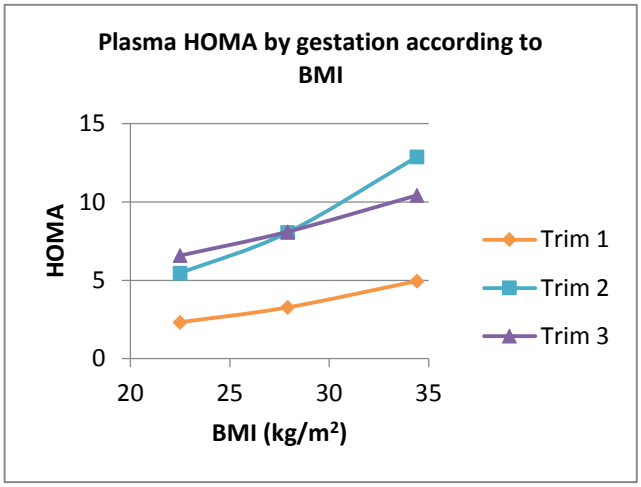

Plasma TAG by gestation according to BMI
Triglyceride (mmol/L)
3.5
3
2.5
2
1.5
1
20
25
30
35
BMI (kg/m²)
Trim 1
Trim 2
Trim 3

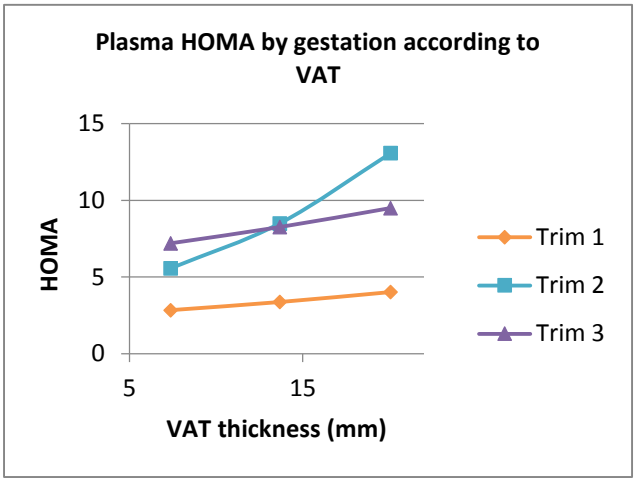

Plasma TAG by gestation according to VAT
Triglyceride (mmol/L)
3.5
3
2.5
2
1.5
1
5
15
VAT thickness (mm)
Trim 1
Trim 2
Trim 3

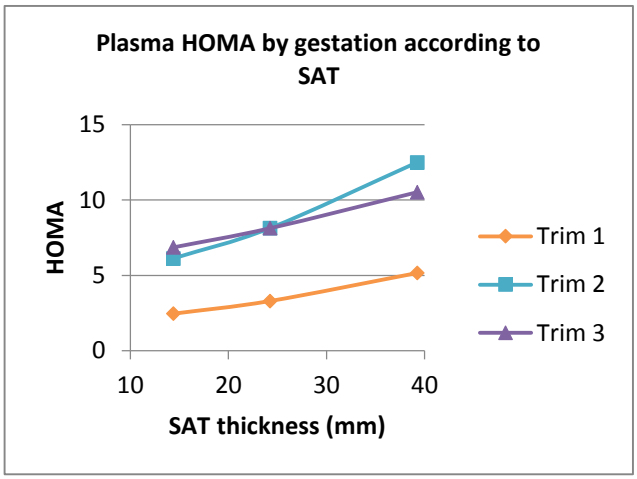

Plasma TAG by gestation according to SAT
Triglyceride (mmol/L)
3.5
3
2.5
2
1.5
1
10
20
30
40
SAT thickness (mm)
Trim 1
Trim 2
Trim 3

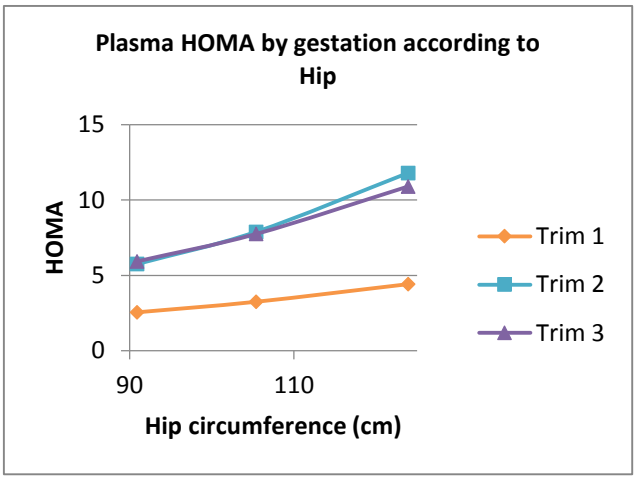

Plasma TAG by gestation according to Hip
Triglyceride (mmol/L)
3.5
3
2.5
2
1.5
1
90
110
Hip circumference (cm)
Trim 1
Trim 2
Trim 3

741
742 **C**


Plasma HOMA by gestation according to BMI
HOMA
15
10
5
0
20
25
30
35
BMI (kg/m²)
Trim 1
Trim 2
Trim 3


Plasma HOMA by gestation according to VAT
HOMA
15
10
5
0
5
15
VAT thickness (mm)
Trim 1
Trim 2
Trim 3


Plasma HOMA by gestation according to SAT
HOMA
15
10
5
0
10
20
30
40
SAT thickness (mm)
Trim 1
Trim 2
Trim 3


Plasma HOMA by gestation according to Hip
HOMA
15
10
5
0
90
110
Hip circumference (cm)
Trim 1
Trim 2
Trim 3

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Supplemental Figure 2. Association between fetal *de novo* lipogenesis and cord blood triglyceride concentrations. Univariate correlation (Pearson's) between cord blood triglyceride concentration (log) and an index of fetal *de novo* lipogenesis (erythrocyte 16:0/18:2 n-6; n=2 data missing) r=-0.44 *P*=0.044.

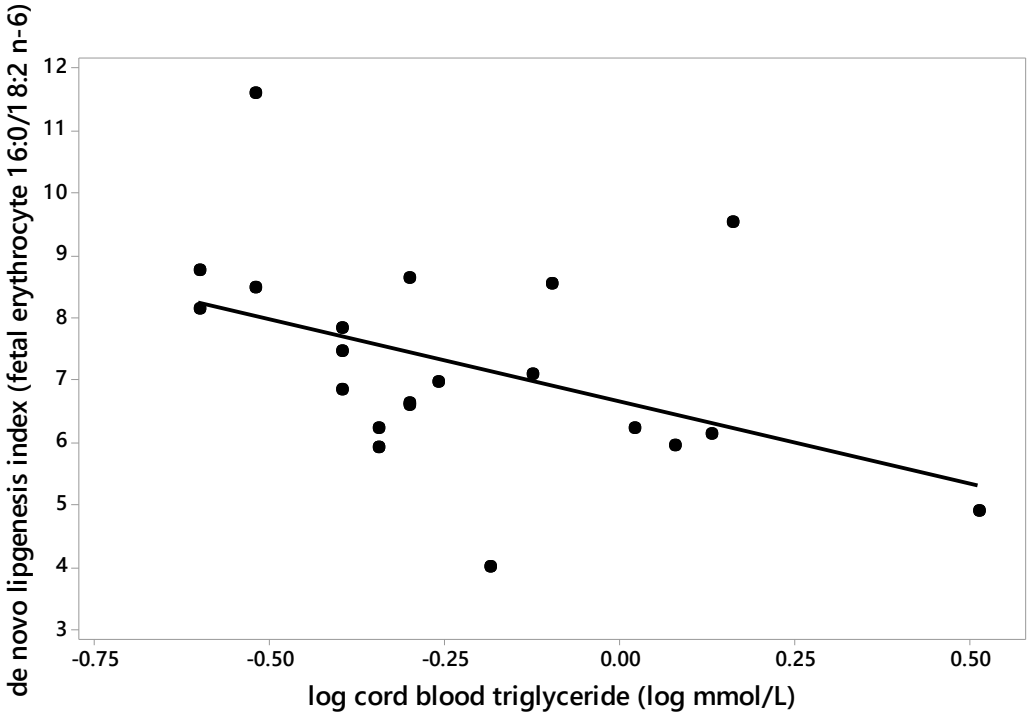

Figure 3