
Clinical Research Article

Mediators Linking Maternal Weight to Birthweight and Neonatal Fat Mass in Healthy Pregnancies

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Abbreviations: ELISA, enzyme-linked immunosorbent assay; FM%, neonatal fat mass percentage; fa, fetal umbilical artery; fv, fetal umbilical vein; GWG, gestational weight gain; HMW, high-molecular weight; pBMI, pregestational body mass index.

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Abstract

Context: Lifestyle interventions have not efficaciously reduced complications caused by maternal weight on fetal growth, requiring insight into explanatory mediators.

Objective: We hypothesized that maternal mediators, including adiponectin, leptin, insulin, and glucose, mediate effects of pregestational BMI (pBMI) and gestational weight gain (GWG) on birthweight and neonatal fat mass percentage (FM%) through placental weight and fetal mediators, including insulin levels (I_{fa}) and venous-arterial glucose difference (ΔG_{fa}). Hypothesized confounders were maternal age, gestational age, and parity.

Methods: A cross-sectional study of healthy mother-offspring-pairs ($n = 165$) applying the 4-vessel *in vivo* sampling method at Oslo University Hospital, Norway. We obtained

pBMI, GWG, birthweight, and placental weight. FM% was available and calculated for a subcohort ($n = 84$). We measured circulating levels of adiponectin, leptin, glucose, and insulin and performed path analysis and traditional mediation analyses based on linear regression models.

Results: The total effect of pBMI and GWG on newborn size was estimated to be 30 g (range, 16–45 g) birthweight and 0.17 FM% (range, 0.04–0.29 FM%) per $\text{kg}\cdot\text{m}^{-2}$ pBMI and 31 g (range, 18–44 g) and 0.24 FM% (range, 0.10–0.37 FM%) per kg GWG. The placental weight was the main mediator, mediating 25-g birthweight and 0.11 FM% per $\text{kg}\cdot\text{m}^{-2}$ pBMI and 25-g birthweight and 0.13 FM% per kg GWG. The maternal mediators mediated a smaller part of the effect of pBMI (3.8-g birthweight and 0.023 FM% per $\text{kg}\cdot\text{m}^{-2}$ pBMI) but not GWG.

Conclusion: Placental weight was the main mediator linking pBMI and GWG to birthweight and FM%. The effect of pBMI, but not GWG, on birthweight and FM%, was also mediated via the maternal and fetal mediators.

Key Words: body mass index, gestational weight gain, adiponectin, leptin, fetal growth, neonatal fat mass

Abnormal fetal growth is a risk factor for obstetrical and neonatal complications and for developing non-communicable diseases later in life (1). Maternal weight status, measured as pregestational body mass index (pBMI) and gestational weight gain (GWG), are associated with fetal growth (1–5). pBMI and GWG are inversely related to each other; nevertheless, they are both independent determinants of fetal growth (1, 2, 5).

Lifestyle interventions during pregnancy to prevent maternal weight-related pregnancy complications and macrosomia, for example, by limiting excess GWG, have not been efficacious (6). This has stimulated interest in developing alternative approaches to prevent fetal overgrowth and adiposity, such as studies of hormone supplementation in animal models (7). However, the specific factors mediating the link between pBMI and GWG on fetal growth are poorly understood in humans (8).

Pregnancy induces profound alterations in maternal metabolism. A prominent change is the development of relative insulin resistance, which is assumed to ensure a stable glucose supply to the fetus (9). Several studies have found a positive association between maternal glucose levels and fetal size (10, 11). According to the Pedersen hypothesis (12), increasing maternal glucose levels lead to a corresponding rise in fetal blood glucose, stimulating fetal insulin secretion. This hypothesis has firm experimental support (13). The maternal-fetal glucose transport is a complex process in which maternal glucose levels are considered the main driving force. In addition, there is evidence that fetal glucose and insulin levels, in an independent and additive manner, affect fetal glucose consumption, and hence fetal growth. Besides stimulating fetal glucose consumption, insulin is also a growth hormone enhancing intrauterine growth (14).

It is becoming increasingly evident that the placenta plays an important and active role in the maternal-fetal interaction, for example, by modifying the mass of glucose transferred from mother to fetus. Placental weight is often used as an indicator of placental functional capacity (13, 15, 16). Previously, including placental weight in multiple linear regression models has been shown to reduce the effect of BMI and GWG on birthweight and neonatal fat mass percentage (FM%) (11, 17, 18). Nevertheless, the role of placental weight in relation to other potential effect mediators has not been studied in detail.

The size of the maternal adipose tissue is a determinant of insulin sensitivity (19). Hence, women who are overweight or obese ($\text{pBMI} \geq 25$) enter pregnancy with greater degrees of insulin resistance than normal-weight women ($\text{pBMI } 18.5 < 25$) (19). Also, obesity and insulin resistance have been associated with the levels of adipokines, that is, adipocyte-derived cytokines with metabolic effects. Two of the most prominent adipokines related to insulin sensitivity are leptin and adiponectin (20). Leptin is associated with insulin resistance, and plasma leptin levels have been positively associated with BMI, also in pregnancy (21, 22). In pregnancy, the placenta contributes to the plasma levels of leptin (22–24). An association between the maternal leptin level and the offspring's fat mass has been observed (21). However, the relationship between maternal leptin levels and birthweight is not settled (20, 25–30). In contrast to leptin, adiponectin is an insulin-sensitizing hormone and inversely associated with BMI, also in pregnant women (3, 8, 22, 31–35). Adiponectin appears to be secreted exclusively from maternal adipose tissue (8, 33, 36–39). The relationship between maternal adiponectin levels and the offspring's birthweight also remains to be clarified (20, 40–42).

The objective of this study was to investigate, *in vivo*, in human pregnancies, the role of potential mediators in the association between maternal weight status, that is, pBMI and GWG and newborn size, that is, birthweight and FM% (17). We included as maternal mediators, the circulating levels of leptin, adiponectin, insulin, and glucose. We hypothesized that these variables would exert their effects on birthweight and FM% by affecting downstream mediators, including 1) placental weight and 2) fetal levels of glucose and insulin (hereafter referred to as the fetal mediators). We performed path and mediation analyses to estimate the direct and indirect effects of pBMI and GWG on birthweight and FM%.

Materials and Methods

Design and Study Population

This was a cross-sectional study conducted at Oslo University Hospital between 2012 and 2016. The study was approved by the regional committee for medical and health research ethics, Southern Norway, 2419/2011. All participants gave written informed consent.

As previously described (15, 43), we invited healthy, nonsmoking women with uncomplicated singleton pregnancies who were scheduled for an elective cesarean delivery to participate. The cesarean deliveries were performed for indications not related to the health of the mother or fetus. Women with only diet-treated gestational diabetes were included ($n = 4$). The exclusion criteria were known intrauterine growth restriction, preexisting morbidity, medication except for levothyroxine, and occasional use of antiallergics, antiemetics, antibiotics, and antacids.

Of the eligible women, 179 were included, from whom maternal arterial blood samples were available in 165 women, and these constituted the main cohort (Fig. 1).

Data Collection

Birthweight, clinical data, blood samples, and placental weight were collected as described (43, 44). In short, the maternal characteristics, including age, parity, socioeconomic status, smoking status, educational level, morbidity, and medication use, were collected at enrollment.

Pregestational weight and height were obtained from antenatal health cards ($n = 153$, 93%), in which all pregnancy-related information throughout pregnancy was recorded, including the women's weight at the time of conception. The pregestational weight documented on these cards is mainly self-reported. On the day of delivery, participating women were weighed on an impedance scale (Tanita Body Composition Analyzer). The placentas were

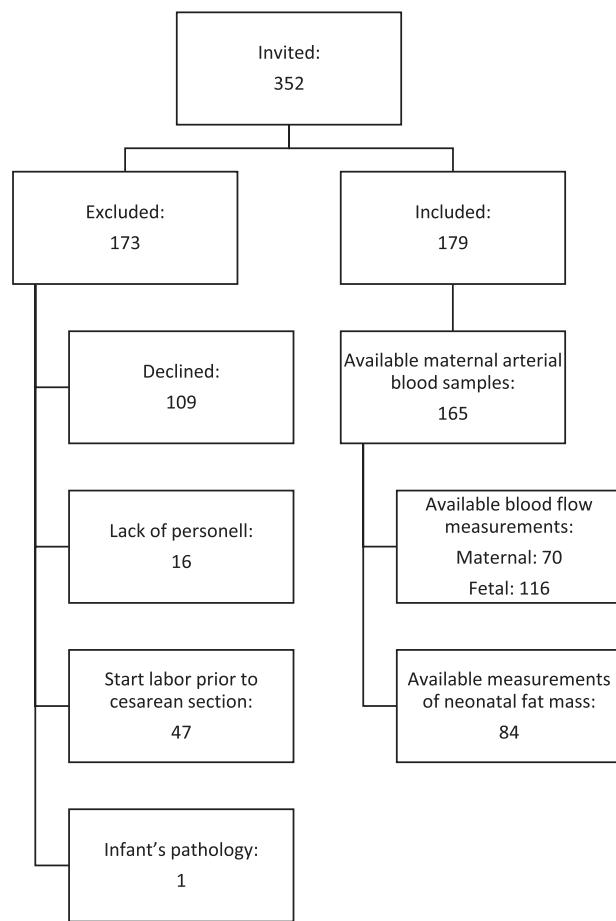


Figure 1. Illustration of the selection of individuals.

weighed untrimmed immediately after delivery. In cases of missing information regarding pregestational weight ($n = 12$), first-trimester weight was used if available ($n = 7$). In cases of missing first-trimester weight ($n = 5$), weight at delivery ($n = 3$), or placental weight ($n = 1$), multiple imputation techniques were applied as described later in "Statistics."

Infant sex, weight, and gestational age at birth were obtained from medical charts. Gestational age was based on ultrasound measures (head circumference) at approximately 18 weeks' gestational age. On the first day postpartum, assessments of skin-fold thicknesses by caliper were performed for a subcohort of 84 newborns. Three caliper measurements per skinfold were performed, and the mean was calculated. All caliper measurements were performed by the same person (M.B.H.). FM% was calculated using the formula from Deierlein et al (45).

The 4-Vessel Sampling Approach

The 4-vessel blood sampling approach (Fig. 2) is described in detail elsewhere (43). The women had been fasting for

more than 8 hours at the time of the cesarean delivery. We collected blood samples from the maternal uterine vein and radial artery, and fetal umbilical vein (fv) and artery (fa). Relative to an ordinary cesarean procedure, the blood sampling procedure extended the operation time by 3 to 5 minutes. No additional bleeding was observed from the sampled uterine vein ($n = 165$) (43). Cesarean delivery was performed under spinal anesthesia (43). The women did not receive glucose infusion during the procedure. The blood samples were centrifuged at 6 °C, 2500 relative centrifugal force for 20 minutes immediately after collection, and the plasma was collected and stored at -80 °C.

Blood Flow Measurements

Blood flow measurements ($\text{mL} \cdot \text{min}^{-1}$) were obtained using Doppler ultrasound ($n = 70$ maternal and 116 fetal individuals) and performed the day of delivery, as previously described (43).

Biochemical Assays

Glucose and insulin

The analyses were performed by an accredited laboratory (Department of Medical Biochemistry, Oslo University Hospital) (44). Glucose was measured using the hexokinase/ glucose-6-phosphate dehydrogenase enzymatic in vitro test (Roche Diagnostics, GLUC3, Glucose

HK Gen3, 05168791 190). Insulin was analyzed using the electrochemiluminescence immunoassay (Roche Diagnostics, Elecsys Insulin, 12017547 122).

Adiponectin

The concentrations of total and high-molecular weight (HMW) adiponectin were determined using an enzyme-linked immunosorbent assay (ELISA) (HMW and total Adiponectin ELISA, ALPCO, 47-ADPHU-E01) according to the manufacturer's instructions. The HMW adiponectin was detected by pretreating the samples with a protease. Protease-treated and -untreated plasma samples from the same individual were analyzed in duplicates and on the same microtiter plate.

The coefficient of variation for the difference between duplicates was 1.27% (0.53%-2.02%) and 1.14% (0.57%-2.08%) for HMW and total adiponectin. The intra-assay coefficients for HMW and total adiponectin were 5.6% and 5.8%. The interassay coefficient for HMW and total adiponectin were 20.2% and 18.4%.

Multiplicative normalization was performed to address the relatively large interassay variation (> 10%). The mean of each interassay control duplet was divided by the mean for all interassay controls, resulting in an individual interassay control ratio for each assay. All adiponectin concentrations were normalized to these ratios.

Owing to collinearity between HMW and total adiponectin levels (Spearman $\rho = 0.937$, $P < .001$), both

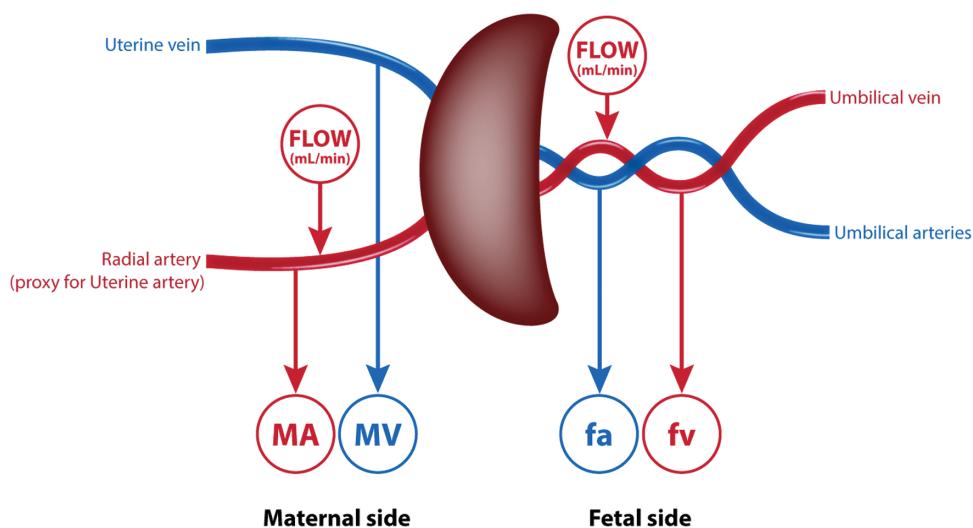


Figure 2. Illustration of the 4-vessels method approach. Blood samples were collected from 4 different blood vessels (indicated as arrows pointing toward circles), which represented 1) blood leading to the uteroplacental unit, that is, the radial artery (MA), which is used as a proxy for the uterine artery; 2) blood returning from the uteroplacental unit, that is, the uterine vein (MV); 3) blood going from the placenta to the fetus, that is, the umbilical vein (fv); and 4) blood returning to the placenta from the fetus, that is, the umbilical arteries (fa). Blood flow measurements (FLOW [mL/min]), indicated by arrows pointing toward blood vessels) were performed on the maternal side, that is, the uterine arteries (bilateral), and the fetal side of the placenta, the fv. Red symbolizes nutrient-rich blood; blue symbolizes nutrient-poor blood. The figure was designed by Øystein Horgmo, University of Oslo.

variables could not be included in the same multivariable models. HMW adiponectin is assumed to be the biologically active isoform in regard to glucose metabolism (46); it was therefore selected to be included in the analyses presented.

Leptin

The concentrations of leptin in maternal plasma were measured in duplicates using an ELISA assay (Quantikine ELISA, R&D Systems, DLP00) according to the manufacturer's instructions. The intra-assay and interassay coefficients were 2.1% and 4.9%, and the coefficient of variation for the difference between duplicates was 2.2% (0.24%-4.44%). Owing to low interassay variation, we did not perform normalization.

Statistics

Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM Corp, released 2016. IBM SPSS Statistics for Windows, version 24.0) and R, version 3.6.3 (47).

Missing data, including pBMI and GWG (on imputing the first-trimester weight, $n = 5$, and $n = 8$, respectively), placental weight ($n = 1$), fetal venous insulin levels ($n = 2$), and fetal venous-arterial glucose difference ($n = 4$), were imputed using the R package Multivariate Imputations

by Chained Equations (MICE) (version 3.9) by predictive mean matching with $n = 20$ multiple imputations (48).

Parity was aggregated into 2 categories, primiparous and multiparous, based on the observation that the greatest impact on birthweight was between the first and second birth (49).

For categorical data, we present the number of cases and percentages of total numbers. For continuous variables, we report medians with interquartile range and minimum and maximum values.

Path analyses

We explored the effects of maternal weight status on birthweight and FM% by path analyses, a statistical technique for investigating hypotheses, in a nonexperimental data set, of the direct and indirect effects of exposure variables on outcome variables based on path diagrams (50, 51). The path diagram (Fig. 3) is a graphical presentation of the hypothesized causal relationship between the variables of interest. In the present cohort, the women's fasting state is taken into account when drawing the path diagram. Square boxes represent observed variables. Single-headed arrows between 2 variables indicate a hypothesized directed causal relationship.

Furthermore, the path analyses were performed as a series of multiple linear regression models (referred to as multivariable models) in which each mediator and outcome

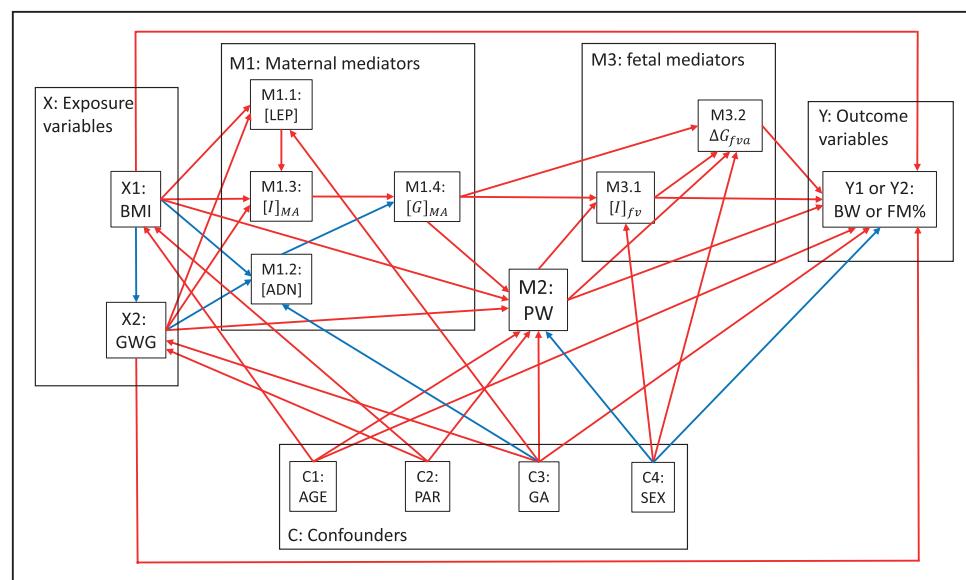


Figure 3. The path diagram presenting the hypothesized relationship between all variables included in the model. Square boxes represent observed variables. Single-headed arrows between 2 variables indicate a hypothesized causal relationship. The women were in a fasting state. Red arrows indicate an hypothesized positive association; blue arrows indicate hypothesized inverse association. ADN, maternal levels of adiponectin; AGE, maternal age; pBMI, maternal pregestational body mass index; FM%, newborn fat mass percentage; LEP, maternal levels of leptin; GA, gestational age; ΔG_{fva} , fetal venous-arterial glucose difference; G_{MA} , maternal arterial levels of glucose; GWG, gestational weight gain; I_{fv} , fetal venous levels of insulin; I_{MA} , maternal arterial levels of insulin; PAR, parity; PW, placental weight; SEX, fetal sex.

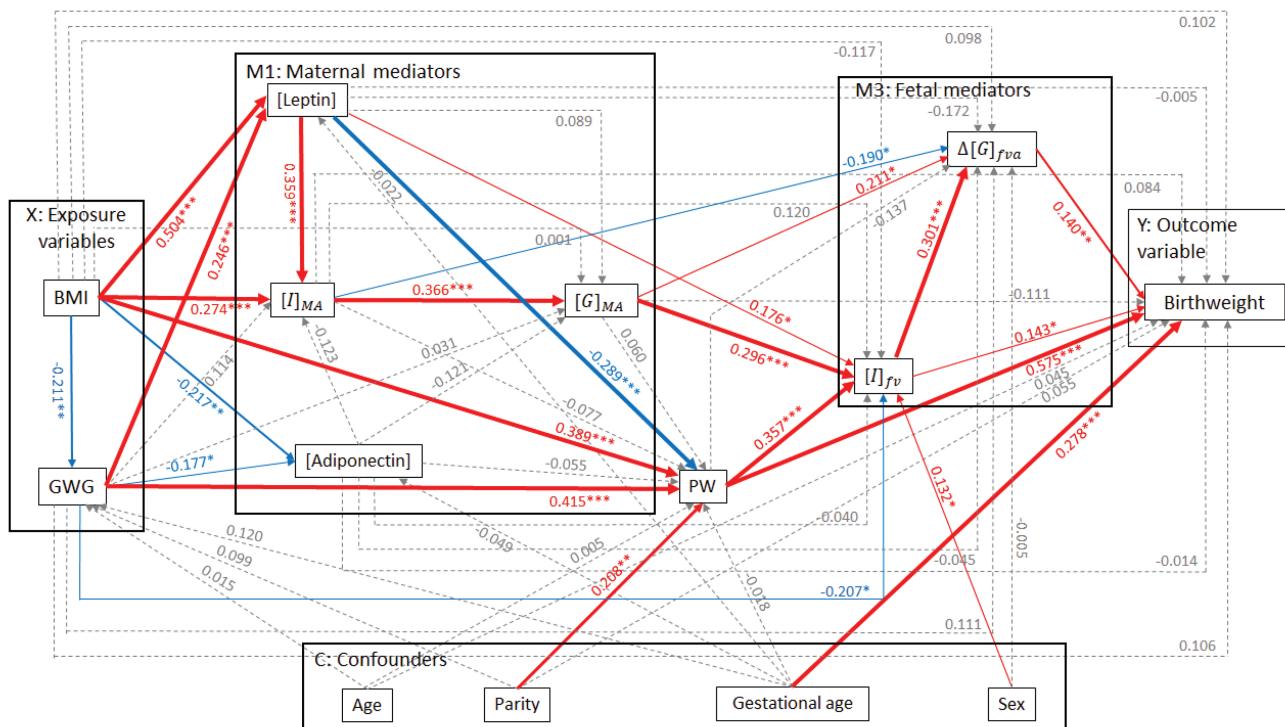


Figure 4. The path analysis with birthweight as the outcome ($n = 165$) presented with standardized β values. Red arrows symbolize a positive association; blue arrows symbolize an inverse association; gray dashed arrows indicate no statistically significant association. Adiponectin, maternal levels of adiponectin; Age, maternal age; BMI, maternal pregestational body mass index; Leptin, maternal levels of leptin; ΔG_{fva} , fetal venous-arterial glucose difference; G_{MA} , maternal arterial levels of glucose; GWG, gestational weight gain; I_fV , fetal venous levels of insulin; I_{MA} , maternal arterial levels of insulin; PW, placental weight; Sex, fetal sex. * $P < .05$; ** $P < .01$; *** $P < .001$.

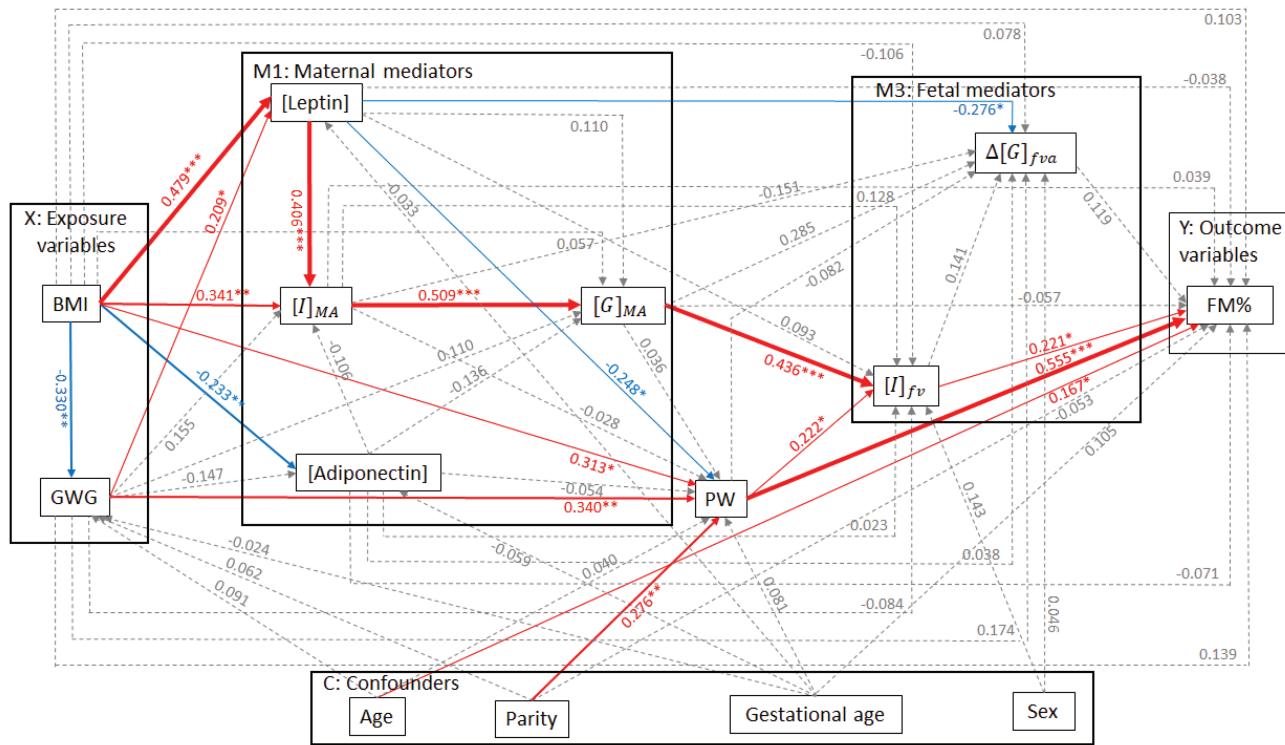


Figure 5. The path analysis with newborn fat mass as the outcome ($n = 84$) presented with standardized β values. Red arrows symbolize a positive association; blue arrows symbolize an inverse association; gray dashed arrows indicate no statistically significant association. Adiponectin, maternal levels of adiponectin; Age, maternal age; BMI, maternal pregestational body mass index; FM%, newborn fat mass percentage; Leptin, maternal levels of leptin; ΔG_{fva} , fetal venous-arterial glucose difference; G_{MA} , maternal arterial levels of glucose; GWG, gestational weight gain; I_fV , fetal venous levels of insulin; I_{MA} , maternal arterial levels of insulin; PW, placental weight; Sex, fetal sex. * $P < .05$; ** $P < .01$; *** $P < .001$.

variable is analyzed by multiple linear regression analysis. The results are illustrated graphically in Figures 4 and 5. All variables included in the multiple linear regression models were also explored in univariate linear regression models, referred to as univariate models.

The variables included in the multiple linear regression models of the path analysis belonged to the following categories: 1) exposure variables, the variables we hypothesized would have an effect on the outcome variable; 2) outcome variables, the variable(s) we hypothesized would depend on the exposure variable; 3) mediators, variables we hypothesized, partially or entirely, would mediate effects of the exposure variable on the outcome variable; and 4) confounders, variables we hypothesized to be associated with both the exposure and outcome or with both the mediator and outcome. We did not include variables that were associated only with either exposure (or mediator) or outcome.

We evaluated the model fit (52). The chi-square test was less than 0.001, indicating a good fit. The comparative fit index was 0.974, which indicates a good fit (> 0.95). The Tucker-Lewis index was 0.911, which indicates an acceptable fit (> 0.90). The root mean squared error approximation was 0.055, which indicates a good fit (< 0.06).

Mediation analyses

Using path analysis, we investigated the paths between maternal weight status (ie, pBMI and GWG) and the offspring's size (ie, birthweight and FM%). Next, we aimed to quantify 1) the total effect of pBMI and GWG on birthweight and FM%; 2) how much of this effect was mediated via the included mediators, that is, the mediated effects of pBMI and GWG on birthweight and FM%; and 3) how much of the effect was independent of the included mediators, that is, the direct effect of pBMI and GWG on birthweight and FM%. For this purpose, we applied traditional mediation analyses (53). In the mediation analysis, the mediators were grouped together (see Fig. 3) in the following categories: 1) maternal mediators; 2) placental weight; and 3) fetal mediators.

The change in birthweight and FM% caused by an increase in pBMI and GWG from the 10th to the 90th percentile was calculated. The estimated effect per unit change of pBMI and GWG on birthweight and FM%, that is, β (95% CI) from the mediation analyses, was multiplied by the difference between the 90th and 10th percentile of the cohort's pBMI ($\Delta pBMI_{(90th - 10th\ percentile)}$) and GWG ($\Delta GWG_{(90th - 10th\ percentile)}$), respectively. The effect of pBMI and GWG on birthweight and FM% mediated via the placental weight (mediated effect_{placental weight}) and the maternal and fetal mediators (mediated effect_{maternal-fetal-mediators}) was calculated by subtracting the respective β values in

the placental-weight-mediator basis model ($\beta_{Placental-weight-mediator\ basis\ model}$) and the maternal-fetal-mediators basis model ($\beta_{Maternal-fetal-mediators\ basis\ model}$) from reference model 2 (mediated effect_{placental weight} = $\Delta\beta_{(Placental-weight-mediator\ basis\ model - Ref.\ 2)}$ and mediated effect_{maternal-fetal-mediators} = $\Delta\beta_{(Maternal-fetal-mediators\ basis\ model - Ref.\ 2)}$). The mediated effect is presented as the percentage of the estimated total effect (reference model 1) ((mediated effect/ $\beta_{Ref\ model\ 1}$) * 100).

Results

Study Population

Clinical and biochemical characteristics of the mother-infant pairs in the main cohort ($n = 165$) and the subcohort with available infant FM% ($n = 84$) are summarized in Tables 1 and 2. The mother-infant pairs represented a healthy population with uncomplicated term pregnancies, and the subcohort was comparable to the main cohort for all characteristics.

Birthweight as Outcome (Main Cohort, $n = 165$)

First, we aimed to identify the paths from maternal pBMI and GWG to birthweight. As described in the following sections, we used path analysis for this purpose, performed as a series of multiple linear regression models, which are presented in Fig. 4. We identified 2 paths:

1. the main path, via placental weight, independent of the maternal mediators
2. a secondary path, via the maternal mediators, depending on the fetal mediators

These 2 paths, including the main finding from the analyses, are presented later in a step-by-step manner.

Second, using mediation analyses, we aimed to quantify the total effects, the mediated effects, and the direct effects of pBMI and GWG on birthweight, as explained in detail later. pBMI and GWG both had a large impact on birthweight (see ref. 1 in Table 3): The analyses estimated that a change in pBMI from 19.3 (the cohort's 10th percentile) to 28.3 (the cohort's 90th percentile) would lead to an increase in birthweight of 273 g (range, 143–403 g). Furthermore, an increase in GWG from 9.5 kg (the cohort's 10th percentile) to 20.8 kg (the cohort's 90th percentile) would lead to an increase in birthweight of 353 g (range, 205–501 g). The direct effect of pBMI and GWG, on adjusting for all mediators, was not statistically significant (see ref. 2 in Table 3), implying that the impact of pBMI and GWG were mainly mediated via the included mediators, that is, the maternal mediators (adiponectin, leptin,

Table 1. Clinical characteristics

Characteristics	Main cohort (n = 165)			Subcohort (n = 84)		
	No. (%)	Median (Q ₁ to Q ₃)	Min to max	No. (%)	Median (Q ₁ to Q ₃)	Min to max
Age, y		36.0 (33.0 to 28.0)	27 to 44		35.5 (32.0 to 38.8)	27 to 44
Higher education, yes	144 (87.3)			74 (88.1)		
Employed, yes	158 (95.8)			82 (97.6)		
Married/partner	160 (97.0)			79 (94.0)		
Parity, nulliparous	41 (24.8)			23 (27.4)		
Gestational age, d		275 (273 to 276)	260 to 294		275 (273 to 276)	264 to 294
In vitro fertilization, yes	13 (7.9)			4 (4.8)		
Smoking, nonsmoker	158 (95.8)			82 (97.6)		
Smoking, stopped in first trimester	7 (4.2)			2 (2.4)		
Gestational diabetes, yes	4 (2.4)			2 (2.4)		
Fasting, h past midnight		10.0 (9 to 11)	8.5 to 17.5		10.0 (9.0 to 11.0)	8.5 to 14.5
pBMI ^a		22.3 (20.8 to 25.1)	17.0 to 47.6		22.0 (20.8 to 25.2)	17.0 to 47.6
Underweight pBMI < 18.5	4 (2.4)			1 (1.3)		
Normal weight, pBMI 18.5-25	114 (71.3)			57 (71.3)		
Overweight, pBMI 25-30	31 (19)			14 (17.5)		
Obese, pBMI ≥ 30	11 (6.8)			8 (10)		
Gestational wt gain, kg ^b		15.0 (13 to 17.8)	-1.2 to 31.0		15.2 (13.1 to 17.5)	-1.2 to 25.7
Offspring's sex, girl	73 (44.2)			38 (45.2)		
Birthweight, g		3558 (3222 to 3826)	2297 to 4955		3608 (3216 to 3822)	2297 to 4430
Small for gestational age, < 10th percentile	24 (14.5)			9(10.7)		
Large for gestational age, > 90th percentile	11 (6.7)			5 (5.9)		
Placental wt, g		613 (523 to 681)	310 to 1115		615 (544 to 677)	310 to 989
FM% ^c					12.5 (10.4 to 14.1)	2.5 to 17.7

Abbreviations: FM%, neonatal fat mass percentage; Max, maximum; Min, minimum; pBMI, pregestational body mass index; Q, quartile.

^an = 160.

^bn = 157.

^cn = 84.

glucose, and insulin), placental weight, and the fetal mediators (insulin and venous-arterial glucose difference). In line with this, the model's R^2 (0.64) was reduced by only less than 0.01 when pBMI and GWG were excluded from the model (see ref. 2 and ΔR^2 (Ref. 2 – model excluding pBMI and GWG) in Table 4). The estimated mediated effects are presented later.

The main pathway via placental weight

This section describes the path from pBMI and GWG to birthweight via the placental weight (see Fig. 4). First, the multivariable model with placental weight as the dependent variable is presented. Thereafter, direct and indirect links between placental weight and birthweight are shown. Finally, the mediated effect of pBMI and GWG on birthweight via the placenta weight is described.

pBMI and GWG were both significantly associated with placental weight ($P < .001$ for both) in the multivariable model in addition to parity ($P = .002$) (see Fig. 4 and Supplementary Table 1 [54]). The placental weight increased by 12.5 g (95% CI, 7.3-17.8 g) per kg·m⁻² pBMI, 12.1 g (95% CI, 7.9-16.2 g) per kg GWG, and 64.7 g (95% CI, 23.1-106.3 g) increase from parity nulliparous to multiparous. Maternal levels of glucose (G_{MA}) were positively but not statistically significantly associated with placental weight in either the univariate or multivariable analyses (see Fig. 4 and Supplementary Table 1 [54]). Maternal leptin levels, on the other hand, were inversely associated with placental weight (see Fig. 4 and Supplementary Table 1 [54]).

Placental weight was the main explanatory variable of birthweight in the multivariable model (see Fig. 4 and

Table 2. Biochemical characteristics

Characteristics	Main cohort (n = 165)		Subcohort (n = 84)		
	Median (Q ₁ to Q ₃)	Min to max	Median (Q ₁ to Q ₃)	Min to max	
Adiponectin, total, µg·mL ⁻¹	5.01 (3.64 to 6.36)	1.43 to 22.72	5.00 (3.62 to 6.08)	1.86 to 22.72	
Adiponectin, HMW, µg·mL ⁻¹	2.66 (1.93 to 3.60)	0.34 to 17.58	2.52 (1.79 to 3.51)	0.54 to 17.58	
Leptin, µg·L ⁻¹	20.3 (11.7 to 28.7)	3.7 to 78.2	19.71 (11.3 to 28.4)	3.7 to 78.2	
Glucose, mmol·L ⁻¹	Radial artery Umbilical vein ^a Δ(umbilical v.a.) ^b	4.48 (4.23 to 4.84) 3.80 (3.50 to 4.07) 0.53 (0.26 to 0.75)	3.52 to 6.56 2.55 to 5.31 -0.54 to 1.36	4.44 (4.31 to 4.80) 3.80 (3.54 to 4.10) 0.54 (0.35 to 0.77)	3.52 to 6.56 2.55 to 5.31 -0.54 to 1.33
Insulin, pmol·L ⁻¹	Radial artery Umbilical vein ^a	57.8 (37.6 to 86.0) 66.5 (41.4 to 90.8)	9.7 to 374.7 15.1 to 283.3	60.4 (41.6 to 91.9) 69.2 (44.6 to 89.7)	15.1 to 374.7 23.7 to 283.3
Flow, mL·min ⁻¹	Uterine artery ^c Umbilical vein	500.5 (362.2 to 615.0) 207.2 (165.1 to 232.2)	181.8 to 1126.1 60.4 to 471.2	502.4 (370.0 to 621.0) 202.0 (176.2 to 238.5)	239.6 to 1126.1 95.7 to 458.1
Flow, mL·min ⁻¹ kg ⁻¹	Umbilical vein ^d	54.1 (47.4 to 64.9)	21.8 to 132.0	59.6 (48.0 to 67.3) ^e	32.4 to 132.0

Abbreviations: HMW, high-molecular weight; Max, maximum; Min, minimum; Q, quartile.

^an = 163.

^bn = 161.

^cn = 60.

^dn = 116.

^en = 55.

Supplementary Table 2 [54]). The effect of placental weight was 2 to 4 times greater than that of other significant explanatory variables: fetal venous-arterial glucose difference (ΔG_{fa}), fetal venous insulin levels (I_{fa}), and gestational age. In line with this, the R^2 was increased by 0.212, an increase of 50% (see The placental-weight-mediator basis model and ΔR^2 (Ref. 2 – Placental-weight-mediator's basis model) in Table 4), on including placental weight in the model. Additionally, placental weight was also indirectly associated with birthweight as it was one of the main explanatory variables of (I_{fa}) (see Fig. 4 and Supplementary Table 3 [54]).

The effect estimates of pBMI and GWG on birthweight were greatly reduced by the inclusion of placental weight (see The placental-weight-mediator basis model vs the mediated effect in Table 3). The estimated mediated effect of pBMI and GWG on birthweight via placental weight implied that if the pBMI and GWG increased from the 10th to 90th percentile, 80% of the birthweight attributed to the increase in pBMI and GWG would be mediated via the placental weight.

The pathway via the maternal mediators

This section presents the path from pBMI and GWG to birthweight via the maternal mediators. The first and second paragraphs focus on adiponectin and leptin. The third paragraph focuses on the maternal levels of insulin (I_{MA}) and glucose (G_{MA}). The fourth paragraph focuses on the links between maternal mediators and birthweight. Finally, the fifth paragraph discloses the estimated mediated effect of pBMI and GWG on birthweight via the maternal mediators.

pBMI and GWG were both inversely associated with maternal levels of adiponectin (see Fig. 4 and Supplementary Table 4 [54]). However, in the downstream path, adiponectin was associated with maternal levels of insulin (I_{MA}) and glucose (G_{MA}) only in the univariate models, but not in the multivariable model (Supplementary Tables 5 and 6 [54]). Moreover, adiponectin was not directly associated with other downstream mediators, including placental weight (see Supplementary Table 1 [54]), I_{fa} (see Supplementary Table 3 [54]), and ΔG_{fa} (see Supplementary Table 7 [54]).

pBMI and GWG were both positively associated with leptin (see Fig. 4 and Supplementary Table 8 [54]). Furthermore, in the downstream path, leptin was associated with I_{MA} (see Fig. 4 and Supplementary Table 5 [54]), but not G_{MA} (see Supplementary Table 6 [54]). Additionally, leptin was positively associated with I_{fa} (see Supplementary Table 3 [54]).

In addition to leptin, pBMI was directly associated with I_{MA} in the multivariable model (see Fig. 4 and Supplementary Table 5 [54]). GWG, however, was not associated with I_{MA} in the univariate or multivariable model. Furthermore, pBMI was associated with G_{MA} in the univariate model but not in the multivariable model, and GWG was not associated with $[G]_{MA}$ in the univariate or multivariable model. This implies that I_{MA} was the only statistically significant explanatory variable of G_{MA} (see Supplementary Table 6 [54]). In the downstream path, G_{MA} was associated both with I_{fa} and ΔG_{fa} .

None of the maternal mediators had an independent direct effect on birthweight in the multivariable model (see

Table 3. Overview of mediation analyses

	Main cohort (n = 165)				Subcohort (n = 84)				Outcome: newborn fat mass %			
	Outcome: birthweight				Outcome: birthweight				Outcome: newborn fat mass %			
	β	P	95% CI	Std β	β	P	95% CI	Std β	β	P	95% CI	Std β
			Lower	Higher			Lower	Higher			Lower	Higher
Reference total effect, ref. 1												
pBMI	30.4	<.001	15.9	44.8	0.281	20.9	.027	2.3	39.4	0.225	0.165	.010
GWG	31.2	<.001	18.1	44.3	0.325	33.2	.001	13.1	53.2	0.341	0.235	.001
Reference direct effect, ref. 2												
pBMI	11.0	.107	-2.4	24.5	0.102	5.0	.552	-11.5	21.4	0.054	0.064	.279
GWG	10.4	.067	-0.7	21.5	0.108	9.4	.246	-6.5	25.3	0.096	0.092	.109
Reference-mediated effect ^a												
pBMI	19.3											
GWG	20.8											
Maternal-fetal-mediators' basis model												
pBMI	14.9	.009	3.7	26.0	0.137	8.3	.244	-5.7	22.3	0.089	0.087	.086
GWG	10.5	.051	-0.05	21.0	0.109	13.9	.078	-1.6	29.4	0.142	0.115	.042
Maternal-fetal-mediators' mediated effect ⁹												
pBMI	3.8											
GWG	0.07											
Placental-weight-mediator basis model												
pBMI	35.5	<.001	19.7	51.4	0.329	23.8	.025	3.0	44.6	0.256	0.176	.012
GWG	35.4	<.001	22.8	47.9	0.369	30.9	.002	11.1	50.7	0.316	0.219	.001
Placental-weight-mediator: mediated effect ^a												
pBMI	24.5											
GWG	25.0											

Reference model 1: The model for calculating the total effect of pBMI and GWG on birthweight, the effect before adjusting for the mediators, included pBMI, GWG, and confounding variables (ie, parity, gestational age, and maternal age). Reference model 2: The model for calculating the direct effect of pBMI and GWG on birthweight and FM%, the effect after adjusting for the included mediators, included pBMI, GWG, confounding variables (as ref. model 1), and the mediators, that is, maternal mediators (adiponectin, leptin, insulin, and glucose), placental weight, and fetal variables (insulin and venous-arterial glucose difference). The reference-mediated effect is the estimated effect of pBMI and GWG on birthweight and FM% that is mediated via the included mediators. This was calculated by subtracting the estimated direct effect from the estimated total effect ($\Delta\beta_{Ref\ model\ 1} - \Delta\beta_{Ref\ model\ 2}$). The maternal-fetal-mediators' basis model was the model used for calculating the effect of pBMI and GWG on birthweight and FM% mediated via the maternal and fetal mediators combined. The maternal-fetal-mediator's basis model estimated the effect of pBMI and GWG on birthweight and FM% before adjusting for the maternal and fetal mediators. The independent variables included in the model were pBMI, GWG, confounding variables (as ref. model 1), and placental weight. The maternal-fetal-mediator's mediated effect was calculated by subtracting the direct effect from the effect estimated from maternal-fetal-mediator's basis model ($\Delta\beta_{Maternal-fetal-mediator's\ basis\ model} - \Delta\beta_{Maternal-fetal-mediator's\ basis\ model - Ref\ model\ 2}$). The placental-weight-mediator's basis model was the model used for calculating the effect of pBMI and GWG on birthweight and FM% mediated via placental weight. The independent variables included in the model were pBMI and GWG, maternal mediators, fetal mediators, and confounding variables (as ref. model 1). The placental-weight-mediator effect was calculated by subtracting the direct effect from the placental-weight-mediator basis model ($\Delta\beta_{Placental-weight-mediator's\ basis\ model} - \Delta\beta_{Placental-weight-mediator's\ basis\ model - Ref\ model\ 2}$).

Abbreviations: FM%, neonatal fat mass percentage; pBMI, pregestational body mass index; GWG, gestational weight gain.
^aAnalyses do not supply 95% CI; however, variation in the data is indicated by the reference and basis models.

Table 4. Overview of the mediation analyses models' R^2 and R^2 -change relative to reference model 2

	Outcome variable: birthweight (n = 165)	Outcome variable: birthweight (n = 84)	Outcome variable: FM% (n = 84)
	R^2	R^2	R^2
Reference model 2	0.641	0.629	0.591
Reference model 1	0.297	0.229	0.221
$\Delta R^2_{(\text{Ref 2} - \text{Ref 1})}$	0.344	0.400	0.371
Maternal-fetal-mediators basis model	0.596	0.580	0.526
$\Delta R^2_{(\text{Ref 2} - \text{Maternal-fetal-mediators' basis model})}$	0.045	0.049	0.065
Placental-weight-mediator basis model	0.430	0.356	0.380
$\Delta R^2_{(\text{Ref 2} - \text{Placental-weight-mediator's basis model})}$	0.211	0.273	0.211
Model excluding pBMI and GWG	0.632	0.619	0.572
$\Delta R^2_{(\text{Ref 2} - \text{model excluding pBMI and GWG})}$	0.009	0.010	0.019

Reference models 1 and 2, the maternal-fetal-mediator's basis model, and the placental-weight-mediator basis model are described in Fig. 3A. The model excluding pBMI and GWG is identical to reference model 2 but without pBMI and GWG, meaning that it included confounding variables (as ref. model 1), maternal mediators, placental weight, and fetal mediators.

Abbreviations: FM%, neonatal fat mass percentage; pBMI, pregestational body mass index; GWG, gestational weight gain.

Fig. 4 and Supplementary Table 2 [54]). However, the maternal mediators, except for adiponectin, were indirectly linked to birthweight, mainly via G_{MA} and its association with I_{fa} and ΔG_{fa} , which both were explanatory variables of birthweight (see Fig. 4, Supplementary Tables 2, 3, and 7 [54]).

The estimated mediated effect of pBMI and GWG on birthweight via the maternal and fetal mediators combined (see Maternal-fetal-mediators: the mediated effect in Table 3) implied that if the pBMI increased from the 10th to 90th percentile (see earlier), 15% of the birthweight attributed to the increase in pBMI was mediated via the maternal and fetal mediators. None of the effects of GWG on birthweight were mediated via the maternal and fetal variables combined. When maternal and fetal mediators were excluded from the model, the R^2 was reduced from 0.636 to 0.589 (see $\Delta R^2_{(\text{Ref. 2} - \text{Maternal-fetal-mediator's basis model})}$ in Table 4), a reduction of 0.045.

Neonatal Fat Mass Percentage (Subcohort, n = 84)

FM% was available for a subcohort of 84 individuals. Therefore, path analysis similar to that of the main cohort was performed in the subcohort, with both FM% and birthweight as outcomes (Fig. 5, Supplementary Figs. 1 and 2, and Supplemental Tables 9-17 [54]). The path-analysis with birthweight as the outcome will briefly be compared as follows to that of the main cohort. After that, the path analysis with birthweight and FM% as outcome variables will shortly be presented. Finally, the results from the mediation analyses will be described.

In the path analysis for the subcohort, with birthweight as the outcome, there were some differences in

the model's R^2 and effect estimates of different variables compared to the main cohort (see Supplementary Fig. 1, and Supplementary Tables 9-16 vs 1 to 8 [54]). However, most of the differences were small and did not affect the conclusions.

Similar to the main cohort, placental weight was the main explanatory variable of FM%, as well as the birthweight in the subcohort (Fig. 5, Supplementary Fig. 1, and Supplementary Tables 10 and 17 [54]). The standardized β of placental weight was 2 to 4 times larger than that of other explanatory variables for both FM% and birthweight. ΔG_{fa} and gestational age were statistically significant explanatory variables of birthweight, whereas I_{fa} and maternal age were statistically significant explanatory variables of FM%.

The mediation analyses (see ref. 1 in Table 3A) implied that a change in pBMI and GWG from the 10th to the 90th percentile (see the second paragraph earlier) would cause an increase of 1.47 FM% (range, 0.36-2.59 FM%) and 2.66 FM% (range, 1.12-4.18 FM%), respectively. In the multivariable model, the direct effect of pBMI and GWG on FM% was not significant (see ref. 2 in Table 3).

The estimated mediated effect of pBMI and GWG on FM% via the placental weight (see Placental-weight-mediator: the mediated effect in Table 3) implied that if the pBMI increased from the 10th to the 90th percentile (see the second paragraph earlier), 68% and 53% of the FM% attributed to the increase in pBMI and GWG, respectively, would be mediated via the placental weight. Finally, the estimated effect of pBMI and GWG on FM% mediated via the maternal and fetal mediators jointly (see Maternal-fetal-mediators: the mediated effect in Table 3) implied that if the pBMI and GWG increased from the 10th to the 90th percentile (see the second paragraph earlier), 14%

and 10% of the FM% attributable to the increase in pBMI and GWG would be mediated via the maternal and fetal mediators, respectively.

Excluded Variables

Blood flow measurements, fetal venous levels of glucose, and fetal sex were considered for the multiple linear regression model of birthweight but were excluded from the final model. The rationale for exclusions is described in the Supplementary manuscript (54).

Discussion

This was a human *in vivo* study applying the 4-vessel sampling method, including 165 healthy mother-fetus pairs. We observed a strong effect of pBMI and GWG on birthweight. Mediation analyses indicated that an increase in pBMI from 19.3 to 28.3 (the cohort's 10th to 90th percentile) would increase the birthweight by 273 g (range, 143-403 g), and an increase in GWG from 9.5 kg to 20.8 kg (the cohort's 10th to 90th percentile) would increase the birthweight by 352 g (range, 205-501 g).

Placental Weight: The Main Mediator of the Effect of Pregestational Body Mass Index and Gestational Weight Gain

Placental weight, independent of the included maternal mediators, was the main mediator of the effect of pBMI and GWG on neonatal size, measured as birthweight or FM% (Fig. 6 and Supplementary Fig. 2 [54]). Approximately 80% of the birthweight attributed to an increase in pBMI and GWG from the 10th to the 90th percentile would be mediated by placental weight.

The significant association between pBMI and GWG and placental weight is in agreement with a recent report from Roland and colleagues (16). However, we did not find a significant mediation effect of G_{MA} on placental weight as we hypothesized based on the work of Roland et al. This difference may reflect differences in 1) the cohort's characteristics, 2) sample size, or 3) study designs, as further discussed later.

In contrast to our hypothesis, maternal leptin levels were inversely associated with placental weight. This finding was in line with observations reported by Schubring et al (26). However, the inverse association between leptin and placental weight may appear contradictory to the observation that pBMI and GWG are positively associated both with leptin and placental weight. From previous studies, obesity has been characterized by hyperleptinemia and leptin resistance (55). Furthermore, lack of or resistance

to leptin due to genetic mutations causes severe obesity, that is, monogenic obesity (56, 57). Treating patients with congenital leptin deficiency with leptin has led to normalization of body weight (56). Moreover, Farley et al (58) found evidence of placental leptin resistance among obese women. The leptin resistance was indicated by a reduced placental expression of leptin receptors, which was accompanied by altered placental function, that is, decreased placental amino acid transport. These studies suggest that resistance to leptin, and not the effect of leptin, is causing obesity and that leptin resistance also could occur in the placenta and affect its function. The observed inverse association between maternal leptin levels and placental weight is compatible with the described previous observations, which imply that increased leptin levels may indicate a relative leptin resistance. From another perspective, leptin, among leptin-sensitive individuals, may stimulate improved placental functionality such as amino acid transport, increasing placental efficiency requiring less placental tissue (59). However, we have preliminary indications from subgroup analyses that moderator effects are involved in the association between leptin and the placenta. Therefore, further investigation is necessary for a full understanding of the relationship between leptin and placental weight.

Our observations suggested that placental weight was the main explanatory variable both of birthweight and FM%, which is in line with other studies by Roland et al (11) and Friis and colleagues (17). Similar to our observations, these studies also reported a strong positive association between placental weight and birthweight and FM%, respectively. Furthermore, our observations indicated that placental weight, independent of other included mediators, was the main mediator of pBMI and GWG in their association with birthweight and FM%. In line with this, Roland et al also reported a substantial reduction in the effect of BMI and GWG on birthweight after adjusting for the placental weight (11). Moreover, Ouyang et al (18) reported that placental weight mediates the effect of prepregnancy obesity and GWG. However, in contrast to the present study, Roland et al and Ouyang and colleagues did not include fetal variables in their analyses. Our analyses show that the majority of the effect of placental weight on birthweight and FM% is independent of its impact on the fetal mediators, that is, I_{fv} and ΔG_{fva} .

The Included Maternal and Fetal Variables Contribute as Mediators in the Association Between Maternal Weight Status and Fetal Size

The analyses suggested that pBMI, but not GWG, mediated some effects on birthweight via the maternal and fetal variables, although considerably less relative to the impact of

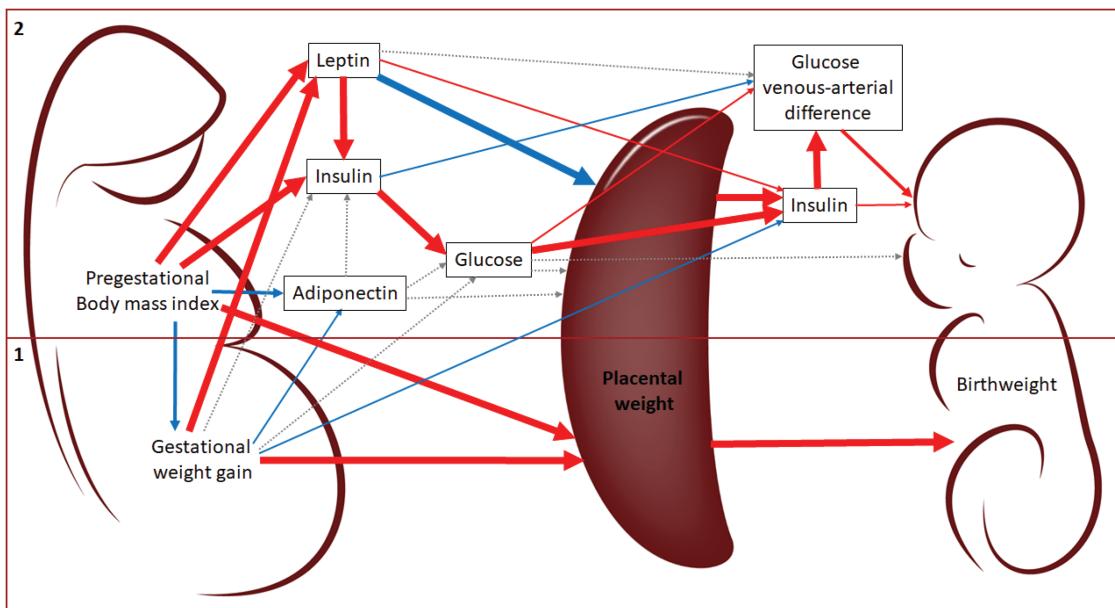


Figure 6. Graphical summary. 1, Placental weight was the main mediator in the association between maternal weight status (ie, pregestational body mass index and gestational weight gain) and offspring's size (ie, birthweight and newborn fat mass percentage [FM%]). 2, Effects of pregestational body mass index, but not gestational weight gain, were also mediated via maternal mediators (ie, leptin, insulin and glucose, but not adiponectin) and fetal mediators (insulin and venous-arterial glucose difference). Red arrows symbolize positive associations; blue arrows symbolize inverse associations; gray dashed arrows indicate no statistically significant associations. The relative effect size is indicated by the thickness of the arrows. The pregnant woman, placenta, and baby are illustrated by Øystein Horgmo, University of Oslo.

placental weight. Fifteen percent of the birthweight attributed to an increase in pBMI from the 10th to the 90th percentile was mediated via the maternal and fetal variables.

In line with our hypothesis, both pBMI and GWG were positively associated with leptin and inversely associated with adiponectin. However, leptin, but not adiponectin, was related to downstream mediators in the path between pBMI and GWG and birthweight, most importantly to I_{MA} (see Fig. 6). Hence, leptin but not adiponectin was a potential mediator of the effects of pBMI and GWG. The positive association between leptin and I_{MA} was in line with our hypothesis and supported the previous findings that leptin modifies maternal insulin sensitivity (23, 46). Adiponectin was associated both with I_{MA} and G_{MA} in univariate analyses, but only borderline significant in multivariable models. One potential explanation is that the significant association in the univariate analyses reflected only adiponectin's association with pBMI, which implies that pBMI was a confounder in the association between adiponectin and I_{MA} and G_{MA} . However, longitudinal studies have shown that maternal adiponectin levels decline across gestation and are lowest at term pregnancy (9, 60). This observation potentially implies that the effects of adiponectin on fetal growth also may decrease throughout pregnancy. Therefore, the impact of term levels of adiponectin on birthweight may be relatively small. Consequently, we cannot exclude the possibility that adiponectin affects fetal growth at earlier stages of pregnancy. In line with this, Jansson et al (3)

observed an association between maternal adiponectin levels and birthweight in early pregnancy but not toward term. Furthermore, our population has a large proportion of healthy normal-weight women (71%) (Table 1). It is therefore possible that a population with a higher proportion of women with overweight, obesity, or gestational diabetes would have led to a different observation.

pBMI, GWG, and leptin were not directly associated with G_{MA} , leaving I_{MA} as the only statistically significant explanatory variable of G_{MA} . This finding is in line with the physiological principle that maternal insulin level was the main determinant of fasting glucose level, mainly influenced by the hepatic glucose output (61). However, pBMI and leptin both were associated with I_{MA} , implying that I_{MA} mediates the effect both of pBMI and leptin on G_{MA} . The association between leptin and I_{MA} was discussed earlier. The association between pBMI and I_{MA} is in line with Catalano (62), who described a positive association between pBMI and insulin resistance throughout pregnancy, associated with increased maternal levels of insulin. In contrast, GWG was not associated with G_{MA} or I_{MA} , indicating that the effect of GWG on birthweight and FM% was not mediated via these 2 variables. In line with this, intervention studies to prevent excessive GWG have not been able to document significant metabolic effects (63).

Finally, none of the included maternal mediators had any significant direct effect on birthweight after adjustments. However, in line with our hypothesis, G_{MA} was a

statistically significant explanatory variable both of I_{fv} and ΔG_{fva} , both of which were significant explanatory variables of birthweight and FM%. The mediation analyses indicated that 15% of the birthweight attributed to the increase in pBMI was mediated via the maternal and fetal mediators. Moreover, excluding the maternal and fetal mediators from reference model 2, which included all variables, reduced the explained variation of birthweight by 4.5%. In other words, although we do not find evidence of a direct link between G_{MA} and birthweight, we do identify an indirect connection with an impact on birthweight. Consistent with this finding, an intervention study of the effect of a low glycemic index diet during the second and third trimesters of pregnancy found that glycemic load was a significant predictor of fetal growth. However, the glycemic load explained less than 1% of the variance, supporting that the link between maternal glucose and fetal growth is complex (63). Furthermore, our observations are in accordance with Pedersen and Hay (12, 14). They both described that the effect of maternal glucose levels on fetal growth was mediated via its regulation of fetal insulin levels and fetal glucose consumption, which both were determinants of fetal growth. In our cohort, the majority of women had levels of glucose and insulin within normal ranges (64). Still, our results indicate that maternal metabolic effects were mediated through fetal glucose and insulin. This underscores the fundamental physiological role of these 2 metabolic factors in fetal growth.

Analysis of Neonatal Fat Mass Percentages

Placental weight was also the main explanatory variable of FM% as it was for birthweight (see Supplementary Fig. 2 [54]). However, I_{fv} but not ΔG_{fva} was associated with FM%. Taking into account the reduced effect of I_{fv} on birthweight in the subcohort, this may imply that I_{fv} is a strong explanatory variable of FM% relative to birthweight.

Women who are overweight or obese give birth to neonates with increased fat mass, even if their glucose tolerance is normal (65). Using the path analysis, we identified an indirect link between maternal glucose levels and fetal fat mass, which is in line with Pedersen and Hay (12, 14). Importantly, our findings indicate that the link between maternal levels of glucose and the neonatal fat mass can also be found in healthy women with normal pregnancies. However, the effect is small compared to the impact of the placental weight.

The Relation Between Maternal Glucose Levels and Placental Weight and Birthweight

In the present study, the association between G_{MA} and birthweight was nonsignificant in the univariate analysis.

These findings contrasted with previous studies, including the Norwegian STORK study and the HAPO study (10, 11). There are at least 3 potential explanations. One explanation is differences in the characteristics of the cohorts. Among others, the present cohort had a large proportion of healthy normal-weight women as well as a large proportion of multiparous women relative to the STORK cohort. Univariable analyses among nulliparous women and multiparous women indicated a potential difference in the effect of G_{MA} on birthweight ($\beta = 220$ vs 54). However, the estimates were not significantly different (95% CI, -209 to 648 vs -97 to 202, and $P = .306$ vs .480). A second explanation is our cohort's relatively lower number of individuals compared to these previous studies. A third explanation is differences in study design, for example, the difference in gestational age or other conditions related to the collection of blood samples.

The Predominant Role of Placental Weight as an Explanatory Factor of Birthweight and Neonatal Fat Mass

The data presented in the present study do not provide any apparent explanations for the effects of placental weight on birthweight, except for the small part of the effect that is mediated via I_{fv} . The villous surface area is closely related to placental weight and may represent a placental property that could determine nutrient delivery and transport (66). Blood flow on both sides of the placenta would expectedly be associated with placental weight. Uterine artery blood flow ($\text{mL} \cdot \text{min}^{-1}$) was, however, not related to birthweight or FM% in the present study, even if the placental weight was not taken into account. This finding may seem surprising; however, it may reflect that the women were healthy with uncomplicated pregnancies without signs of placental dysfunction. On the fetal side, umbilical blood flow ($\text{mL} \cdot \text{min}^{-1}$) per kilogram birthweight was associated with birthweight prior to but not after adjustment for placental weight, but was not associated with FM% (see Supplementary manuscript [54]). Still, the fetoplacental blood flow, as well as the syncytiotrophoblastic surface area, may both be placental properties that determine birthweight and FM%. In addition and importantly, the placenta's own energy consumption and metabolism may vary and affect fetal energy delivery, as we have previously reported (15).

Strengths and Limitations

The invasive human *in vivo* sampling allowed detailed studies of potential mediators linking maternal weight variables to birthweight and neonatal fat mass in the human. The 4-vessel method is logistically challenging. From this perspective, our sample size is large, and it provides

potentially unique insight into the links between maternal weight and fetal growth.

Still, the cohort size did imply limitations regarding analyses, for example, we did not have the opportunity to perform interaction analyses. Therefore, we were unable to separate the path analysis based on pBMI categories, GWG categories, parity, or fetal sex to investigate potential interaction effects (see the section on Fetal sex in the Supplementary manuscript [54]). This is one example of how the sample size limitation was carefully taken into account while designing the models and analyses (see the section on Blood flow and Fetal sex in the Supplementary manuscript [54]). Moreover, it also illustrates how we restricted the complexity of the models to avoid overfitting, in addition to allowing only linear associations. We also carefully selected the variables to be included and kept only those considered essential to address the objective of the study. For examples, see the Supplementary manuscript (54). However, the sample size did have some trade-offs, including an impact on the precision of the effect-estimates. Therefore, we also investigated how the variables of interest affected the coefficient of determination (R^2), which is a more robust statistical measurement, in addition to considering the estimated effect using β (95% CI), standardized β , and P value. Nevertheless, as discussed earlier, the sample size may explain the discrepancies between our study and previous studies regarding the association between maternal glucose and placental weight and birthweight. Moreover, a sample size that allowed interaction analyses could also have clarified whether the result discrepancies were conditioned by the cohort's characteristics. These may be important aspects to consider in future cohort studies.

We conducted a cross-sectional study of term healthy pregnant women. Therefore, our results are not necessarily valid for other stages of pregnancy.

Furthermore, the traditional mediation approach that we used has some limitations. For instance, we observed that the estimated effect mediated via the placental weight was larger than the estimated effect mediated via all included mediators, that is, estimated from a multivariable model, which also encompasses placental weight. One potential explanation for this finding is violations of the assumptions of the mediation analysis, for example, that all relationships can be represented by linear regression and that all confounders are observed and included in the regression models (53). Moreover, the individual mediators are not necessarily independent; they may act as moderators of each other's effect, that is, mediator-mediator-interaction, which is not taken into account in the traditional mediation approach. For example, the inverse association between leptin and placental weight may partially explain why the effect mediated via placental weight was larger than the effect mediated via all included mediators.

The pregestational weight in the antenatal health cards was mainly self-reported; therefore, there is a risk of reporting error (67). However, self-reported weight data in antenatal health cards are regarded as reliable and acceptable as weight references (67). Also, the use of BMI rather than the weight itself reduces the weight error (67).

Conclusions

Placental weight was the main explanatory variable of birthweight and FM% and the predominant mediator of the association between maternal weight status and fetal size. Our findings support that maternal glucose level regulate fetal growth through fetal insulin level and glucose. Furthermore, although pBMI and GWG both were explanatory variables of adiponectin, adiponectin did not act as a mediator of their effect on fetal growth at term. Our observations of an association between maternal leptin and insulin levels support that leptin modifies maternal insulin sensitivity. Furthermore, maternal insulin level was the only explanatory variable of maternal glucose level, in line with the physiological principle that insulin is the main determinant of hepatic glucose output during a fasting state. Our observations indicate that fetal insulin level is more important than fetal venous-arterial glucose difference for FM%. Finally, pBMI and GWG appeared to mediate their effects via separate pathways: The effect of pBMI, but not GWG, was partially mediated via the maternal and fetal mediators. This observation implies that pBMI, but not GWG, has an impact on maternal insulin sensitivity. Placental weight was the only identified mediator linking GWG to birthweight.

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Data Availability: Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality and in accordance with EU General Data Protection Regulation requirements. The corresponding author will, on request, detail the restrictions and any conditions under which access to some data may be provided.

References

- Henriksen T. The macrosomic fetus: a challenge in current obstetrics. *Acta Obstet Gynecol Scand*. 2008;87(2):134-145.
- Goldstein RF, Abell SK, Ranasinha S, et al. Gestational weight gain across continents and ethnicity: systematic review and meta-analysis of maternal and infant outcomes in more than one million women. *BMC Med*. 2018;16(1):153.
- Jansson N, Nilsfelt A, Gellerstedt M, et al. Maternal hormones linking maternal body mass index and dietary intake to birth weight. *Am J Clin Nutr*. 2008;87(6):1743-1749.
- Heslehurst N, Rankin J, Wilkinson JR, Summerbell CD. A nationally representative study of maternal obesity in England, UK: trends in incidence and demographic inequalities in 619 323 births, 1989-2007. *Int J Obes (Lond)*. 2009;34(3):420-428.
- National Research Council 2009. *Weight Gain During Pregnancy: Reexamining the Guidelines*. The National Academies Press; 2009:1-843.
- Sagedal LR, Øverby NC, Bere E, et al. Lifestyle intervention to limit gestational weight gain: the Norwegian Fit for Delivery randomised controlled trial. *BJOG*. 2017;124(1):97-109.
- Aye IL, Rosario FJ, Powell TL, Jansson T. Adiponectin supplementation in pregnant mice prevents the adverse effects of maternal obesity on placental function and fetal growth. *Proc Natl Acad Sci U S A*. 2015;112(41):12858-12863.
- Aye ILMH, Powell TL, Jansson T. Review: Adiponectin—the missing link between maternal adiposity, placental transport and fetal growth? *Placenta*. 2013;34(Suppl):S40-S45.
- Fuglsang J, Skjaerbaek C, Frydryk J, Flyvbjerg A, Ovesen P. A longitudinal study of serum adiponectin during normal pregnancy. *BJOG*. 2006;113(1):110-113.
- Metzger BE, Lowe LP, Dyer AR, et al. Hyperglycemia and adverse pregnancy outcomes. *N Engl J Med*. 2008;358(19):1991-2002.
- Roland MC, Friis CM, Voldner N, et al. Fetal growth versus birthweight: the role of placenta versus other determinants. *PLoS One*. 2012;7(6):e39324.
- Pedersen J. Weight and length at birth of infants of diabetic mothers. *Acta Endocrinol (Copenh)*. 1954;16(4):330-342.
- Hay WW Jr. Placental-fetal glucose exchange and fetal glucose metabolism. *Trans Am Clin Climatol Assoc*. 2006;117:321-339; discussion 339.
- Hay WW. Development of the fetus carbohydrate and lipid metabolism. In: Walker WA, Watkins JB, Duggan C, eds. *Nutrition in Pediatrics Basic Science and Clinical Application*. 3rd ed. BC Decker Inc; 2003:449-470.
- Michelsen TM, Holme AM, Holm MB, et al. Uteroplacental glucose uptake and fetal glucose consumption: a quantitative study in human pregnancies. *J Clin Endocrinol Metab*. 2019;104(3):873-882.
- Roland MC, Friis CM, Godang K, Bollerslev J, Haugen G, Henriksen T. Maternal factors associated with fetal growth and birthweight are independent determinants of placental weight and exhibit differential effects by fetal sex. *PLoS One*. 2014;9(2):e87303.
- Friis CM, Qvigstad E, Paasche Roland MC, et al. Newborn body fat: associations with maternal metabolic state and placental size. *PLoS One*. 2013;8(2):e57467.
- Ouyang F, Parker M, Cerdá S, et al. Placental weight mediates the effects of prenatal factors on fetal growth: the extent differs by preterm status. *Obesity (Silver Spring)*. 2013;21(3):609-620.
- Catalano PM, Shankar K. Obesity and pregnancy: mechanisms of short term and long term adverse consequences for mother and child. *BMJ*. 2017;356:j1.
- Lekva T, Roland MCP, Michelsen AE, et al. Large reduction in adiponectin during pregnancy is associated with large-for-gestational-age newborns. *J Clin Endocrinol Metab*. 2017;102(7):2552-2559.
- Josefson JL, Zeiss DM, Rademaker AW, Metzger BE. Maternal leptin predicts adiposity of the neonate. *Horm Res Paediatr*. 2014;81(1):13-19.
- Lindheim LC. *The Role of Placental Hormones in the Regulation of Maternal Metabolism During Pregnancy*. Diploma Thesis, Department of Obstetrics and Gynecology, Medical University of Graz; 2012.
- Tessier DR, Ferraro ZM, Gruslin A. Role of leptin in pregnancy: consequences of maternal obesity. *Placenta*. 2013;34(3):205-211.
- Masuzaki H, Ogawa Y, Sagawa N, et al. Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med*. 1997;3(9):1029-1033.
- Hassink SG, de Lancey E, Sheslow DV, et al. Placental leptin: an important new growth factor in intrauterine and neonatal development? *Pediatrics*. 1997;100(1):E1.
- Schubring C, Kiess W, Englano P, et al. Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. *J Clin Endocrinol Metab*. 1997;82(5):1480-1483.
- Helland IB, Reseland JE, Saugstad OD, Drevon CA. Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. *Pediatrics*. 1998;101(3):E12.
- Schubring C, Englano P, Siebler T, et al. Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. *Horm Res*. 1998;50(5):276-283.
- Tamura T, Goldenberg RL, Johnston KE, Cliver SP. Serum leptin concentrations during pregnancy and their relationship to fetal growth. *Obstet Gynecol*. 1998;91(3):389-395.
- Czarnobay SA, Kroll C, Schultz LF, Malinovski J, de Barros Silva Mastroeni SS, Mastroeni MF. Predictors of excess birth weight in Brazil: a systematic review. *J Pediatr (Rio J)*. 2019;95(2):128-154.
- Ruan H, Dong LQ. Adiponectin signaling and function in insulin target tissues. *J Mol Cell Biol*. 2016;8(2):101-109.

32. Duval F, Do Santos E, Poidatz D, et al. Adiponectin inhibits nutrient transporters and promotes apoptosis in human villous cytotrophoblasts: involvement in the control of fetal growth. *Biol Reprod.* 2016;94(5):111.
33. Haghjac M, Basu S, Presley L, Serre D, Catalano PM, Hauguel-de Mouzon S. Patterns of adiponectin expression in term pregnancy: impact of obesity. *J Clin Endocrinol Metab.* 2014;99(9):3427-3434.
34. Cnop M, Havel PJ, Utzschneider KM, et al. Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia.* 2003;46(4):459-469.
35. Fisher FM, McTernan PG, Valsamakis G, et al. Differences in adiponectin protein expression: effect of fat depots and type 2 diabetic status. *Horm Metab Res.* 2002;34(11-12):650-654.
36. McDonald EA, Wolfe MW. Adiponectin attenuation of endocrine function within human term trophoblast cells. *Endocrinology.* 2009;150(9):4358-4365.
37. Corbetta S, Bulfamante G, Cortelazzi D, et al. Adiponectin expression in human fetal tissues during mid- and late gestation. *J Clin Endocrinol Metab.* 2005;90(4):2397-2402.
38. Pinar H, Basu S, Hotmire K, et al. High molecular mass multimer complexes and vascular expression contribute to high adiponectin in the fetus. *J Clin Endocrinol Metab.* 2008;93(7):2885-2890.
39. Ramsay JE, Jamieson N, Greer IA, Sattar N. Paradoxical elevation in adiponectin concentrations in women with preeclampsia. *Hypertension.* 2003;42(5):891-894.
40. Horosz E, Bomba-Opon DA, Szymanska M, Wielgos M. Third trimester plasma adiponectin and leptin in gestational diabetes and normal pregnancies. *Diabetes Res Clin Pract.* 2011;93(3):350-356.
41. Perichart-Perera O, Muñoz-Manrique C, Reyes-López A, Tolentino-Dolores M, Espino Y Sosa S, Ramírez-González MC. Metabolic markers during pregnancy and their association with maternal and newborn weight status. *PLoS One.* 2017;12(7):e0180874.
42. Ahlsson F, Diderholm B, Ewald U, et al. Adipokines and their relation to maternal energy substrate production, insulin resistance and fetal size. *Eur J Obstet Gynecol Reprod Biol.* 2013;168(1):26-29.
43. Holme AM, Holm MB, Roland MCP, et al. The 4-vessel sampling approach to integrative studies of human placental physiology in vivo. *J Vis Exp.* 2017;(126):55847.
44. Holme AM, Roland MC, Lorentzen B, Michelsen TM, Henriksen T. Placental glucose transfer: a human in vivo study. *PLoS One.* 2015;10(2):e0117084.
45. Deierlein AL, Thornton J, Hull H, Paley C, Gallagher D. An anthropometric model to estimate neonatal fat mass using air displacement plethysmography. *Nutr Metab (Lond).* 2012;9:21.
46. Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med.* 2008;14(11-12):741-751.
47. R. Core Team. *R: A Language and Environment for Statistical Computing.* Vienna, Austria: R Foundation for Statistical Computing. 2020. Accessed February 2020. <https://www.R-project.org>
48. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in R. *J Stat Softw.* 2011;45(3):1-67.
49. Roland MC. *Fetal Growth: The Role of Maternal Factors and Placenta.* Dissertation. Faculty of Medicine, University of Oslo; 2014:1-82.
50. Stage FK, Carter HC, Nora A. Path analysis: an introduction and analysis of a decade of research. *J Educ Res.* 2004;98(1):5-13.
51. Diouf I, Botton J, Charles MA, et al; EDEN Study Group. Specific role of maternal weight change in the first trimester of pregnancy on birth size. *Matern Child Nutr.* 2014;10(3):315-326.
52. Hu L-T, Bentler PM. Cutoff criteria for fit indexes in covariance structure analysis: conventional criteria versus new alternatives. *Struct Equ Modeling.* 1999;6(1):1-55.
53. VanderWeele TJ, ed. Mediation: Introduction and Regression-Based Approaches. In: *Explanation in Causal Inference—Methods for Mediation and Interaction.* Oxford University Press; 2015.
54. Kristiansen O, Zucknick M, Reine TM, et al. *Kristiansen_supplemental_materials_#jc.2020_03086.* Deposited February 11, 2021. Mendeley Data Repository. <http://dx.doi.org/10.17632/p546xcmthr.1>
55. Biesiada LA, Głowacka E, Krekora M, Sobantka S, Krococka A, Krasomski G. The impact of excessive maternal weight on the nutritional status of the fetus - the role of leptin. *Arch Med Sci.* 2016;12(2):394-401.
56. Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG, Mantzoros CS. Leptin in human physiology and therapeutics. *Front Neuroendocrinol.* 2010;31(3):377-393.
57. Huvenne H, Dubern B, Clément K, Poitou C. Rare genetic forms of obesity: clinical approach and current treatments in 2016. *Obes Facts.* 2016;9(3):158-173.
58. Farley DM, Choi J, Dudley DJ, et al. Placental amino acid transport and placental leptin resistance in pregnancies complicated by maternal obesity. *Placenta.* 2010;31(8):718-724.
59. Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J.* 2009;23(1):271-278.
60. Eriksson B, Löf M, Olausson H, Forsum E. Body fat, insulin resistance, energy expenditure and serum concentrations of leptin, adiponectin and resistin before, during and after pregnancy in healthy Swedish women. *Br J Nutr.* 2010;103(1):50-57.
61. Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care.* 2004;27(6):1487-1495.
62. Catalano PM. Obesity, insulin resistance, and pregnancy outcome. *Reproduction.* 2010;140(3):365-371.
63. Catalano P, deMouzon SH. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. *Int J Obes (Lond).* 2015;39(4):642-649.
64. Catalano PM. Carbohydrate metabolism and gestational diabetes. *Clin Obstet Gynecol.* 1994;37(1):25-38.
65. Sewell MF, Huston-Presley L, Super DM, Catalano P. Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol.* 2006;195(4):1100-1103.
66. Aherne W. A weight relationship between the human foetus and placenta. *Neonatology.* 1966;10(3-4):113-118.
67. Headen I, Cohen AK, Mujahid M, Abrams B. The accuracy of self-reported pregnancy-related weight: a systematic review. *Obes Rev.* 2017;18(3):350-369.