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Obesity

Maternal BMI, Gestational Diabetes, and Weight Gain in Relation to Childhood Obesity: The Mediation Effect of Placental Weight

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Objective: High prepregnancy body mass index (BMI), excessive gestational weight gain (GWG), and gestational diabetes mellitus (GDM) are associated with the risk of childhood obesity. This study aims to examine the extent to which these effects may be mediated through the placenta.

Methods: Data included 33,893 mothers and their singleton infants from birth to 7 years old (total 154,590 visits) in the Collaborative Perinatal Project, a U.S. multicenter prospective cohort study from 1959 to 1976. The placentas were weighed after removing cord and membranes. We performed sequential generalized estimating equation-linear models excluding and including placental weight to evaluate its mediation effect.

Results: In this population, 21.7% of mothers had overweight or obesity, 17.3% had excessive GWG, and 350 (1%) had diagnosed GDM; in addition, 7.2% children had obesity. After adjustment for prepregnancy BMI and other covariates, childhood BMI was 0.23 (95% CI: 0.05, 0.40) kg/m^2 higher for children born to mothers with GDM versus those without GDM. Inclusion of placental weight in the model attenuated the association by 52% to 0.11 (95% CI: −0.06, 0.28) and similarly attenuated the associations with childhood BMI for GWG by 25% and maternal prepregnancy BMI by 17%.

Conclusions: Placental weight partly mediates the effects of prepregnancy BMI, excessive GWG, and GDM on childhood BMI.

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Introduction

Childhood obesity has reached epidemic levels in many parts of the world (1). It confers significant risks for diabetes and cardiovascular disease in adulthood (2). Childhood obesity has its root before birth. Maternal obesity, excessive gestational weight gain (GWG), and gestational diabetes mellitus (GDM), with prevalence as high as 20%, 28%, and 5.8%, respectively, in the United States (3-5), are well-recognized prenatal determinants of childhood obesity (6-9). Children of mothers with these prenatal factors are more likely to have obesity (6,8,10). To prevent the lifelong consequences of obesity, it is important to understand these modifiable prenatal determinants of obesity (maternal body mass index [BMI] at the start of pregnancy, GWG, and GDM) and mechanisms by which these factors contribute to offspring obesity.

The placenta serves as a critical organ that transports nutrients from the mother to fetus (11) and is the interface between the mother and fetus (12). Prepregnancy obesity, excessive GWG, and GDM reflect maternal "over-nutrition" (13,14) and have been associated with

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larger placental size (15-18). The placenta plays a key role in control of fetal growth (19). Significantly, smaller placenta has been observed in pregnancies with intrauterine growth restriction (20).

Our recent study indicated that placental weight mediated the associations between these prenatal factors and infants large for gestational age (LGA) (15), which itself is a predictor of childhood obesity. Previous studies have also demonstrated that placental weight is associated with offspring hypertension and cardiovascular diseases during childhood/adulthood, which suggests the long-term role of placenta in fetal programming of chronic diseases (21). This evidence raises the possibility that there is an intermediate effect of placental weight in the association between prenatal factors (prepregnancy obesity, GWG, and GDM) and childhood obesity (the directed acyclic graph is shown in Supporting Information Figure S1). However, this hypothesis has not yet been examined. Neither is it known whether there is an impact of placental size on childhood weight status, directly, through birth weight, or both.

The objectives of this study were to examine the extent to which placental weight mediates the effects of prepregnancy BMI, GWG, and GDM on childhood obesity. We used data from the U.S. Collaborative Perinatal Project (CPP), a multicenter prospective cohort study.

Methods

Study population

We used data from the CPP (22,23), which was conducted at 12 major medical centers in the United States. It was originally designed to identify potentially causal relationships between perinatal events and childhood cerebral palsy up to age 7 years (22,23). The study population and methods are described elsewhere (22,23). Briefly, women who had their prenatal care at 12 medical centers were enrolled as early as possible in pregnancy in 1959-1965. Each center had its own sampling frame, but they all established and followed extensive procedures of unbiased selection and enrollment. No internal selection bias was found within each center, and the findings from CPP should be representative to the general U.S. population of pregnant women of that era (22,23). The live-born infants of participants were examined at delivery by study nurses and followed up from birth through early school age at 4, 8, and 12 months and 3, 4, and 7 years during 1959-1974. The follow-up rate of these children was 88% at 1 year, 75% at 4 years, and 79% at 7 years of age (23). For this analysis, we excluded 56 women who had diabetes before pregnancy (Supporting Information Figure S2). This study included 34,413 pairs of mothers and their singleton infants delivered at gestational ages of 28-42 weeks with complete data on three prenatal variables (prepregnancy BMI, GWG, and GDM), placental weight, birth weight, and childhood postnatal follow-up measures ($n = 154{,}590$) of height and weight up to 7 years of age (flowchart of participant selection—Supporting Information Figure S2). All 154,590 postnatal follow-up measures were included in the analysis.

Main study variables

Prepregnancy obesity. Maternal prepregnancy BMI (kg/m^2) was calculated by self-reported prepregnancy weight and measured height which were obtained during the initial prenatal examination by trained study staff and health professionals. Prepregnancy BMI was categorized as underweight (<18.5), normal weight (18.5-24.9), overweight (25.0-29.9), and obese (≥30.0) (24).

GWG. GWG was calculated as the difference between the final measured weight within 3 weeks of delivery (recorded under the supervision of obstetricians) and self-reported prepregnancy weight. We categorized GWG into inadequate, adequate, and excessive GWG by week of gestation according to the 2009 Institute of Medicine (IOM) guidelines, with correction for shortened gestational length as indicated per IOM guidelines (24). For example, if a normal-weight woman delivered at 33 weeks, the "adequate" weight gain is between $1.1 + (33 - 13) \times 0.8 = 17.1$ and $4.4 + (33 - 13) \times 1 = 24.4$ pounds. Those below 17.1 pounds (i.e., 7.76 kg) and above 24.4 pounds (i.e., 11.07 kg) were classified as "inadequate" and "excessive," respectively. In addition, we calculated a weekly GWG (100 g/week), as a continuous measure, using the formula: weekly GWG (100 g/week) = 10 × GWG (kg)/gestational age (weeks).

GDM. The presence or absence of GDM was based on the recorded clinical diagnosis in the medical record. During the study reference period, the 100 g, 3-h oral glucose tolerance test (OGTT) was used in the diagnosis of GDM as two or more values exceeding the following cutoffs: fasting 5.8 mmol/l, 1-h 10.6 mmol/l, 2-h 9.2 mmol/l, 3-h 8.1 mmol/l (25,26). Most women with GDM were treated by dietary intervention and then insulin if needed in the control of hyperglycemia (26).

Placental weight and shape. The fresh placentas were weighed after removing cord and membranes to the nearest 10 g by pathology staff. We calculated placental weight *z*-score as placental weight standardized by the mean and SD of placental weight in each stratum of the corresponding (infant) sex and gestational week, analyzed as a continuous variable. We also classified placental weight into five groups based on sex- and gestational age-specific quintiles: the lowest (Q1) to highest quintile (Q5) of placental weight in each stratum of gestational week, respectively, in male and female infants (15).

The shapes of the placenta were examined by pathology staff and classified as (1) round or oval and (2) other shapes which included bipartite, tripartite, succenturiate, membranous placenta, crescent shaped, and irregular shapes.

Placental ovality was measured by the difference between the largest and smallest diameters (27).

Main outcomes—weight status in infancy/childhood

Main measure of weight status in childhood was BMI for all children. Infants were weighed nude (without diapers) through 20 months. Thereafter, light underpants and socks may have been worn. Recumbent length was used through 20 months; thereafter, standing height was measured.

Childhood obesity was defined as sex-specific weight-for-length ≥ 95th percentile for children <2 years of age; and sex- and age-specific BMI ≥ 95th percentile for children ≥2 to 7 years of age

TABLE 1 Characteristics of study participants by sex- and gestational age-specific quintile of placental weight

	Placental weight quintile					
	Q1—lowest (*N* = 6746)	Q2 (*N* = 6836)	Q3 (*N* = 6778)	Q4 (*N* = 6803)	Q5—highest (*N* = 6730)	*P* value
			Mean ± SD			
Placental measures						
Placental weight (g)	320 ± 38	384 ± 22	428 ± 22	477 ± 24	574 ± 66	<0.0001
Largest-smallest diameter (cm)	2.4 ±1.9	2.5 ± 2.0	2.6 ± 2.0	2.6 ± 2.0	2.8 ± 2.2	<0.0001
Placental area (cm^2)	206.8 ± 39.9	230.7 ± 39.7	245.6 ± 41.0	260.4 ± 44.6	288.7 ± 52.7	<0.0001
Placental shape, oval or round (%)	96.9	96.4	96.3	95.7	95.2	<0.0001
Maternal factors						
Age (years)	23.6 ± 5.9	23.8 ± 5.8	24.2 ± 6.0	24.4 ± 5.9	25.1 ± 6.1	<0.0001
Height (cm)	160.3 ± 7.0	160.5 ± 6.8	160.9 ± 6.8	161.3 ± 6.8	161.6 ± 6.7	<0.0001
Weight (kg)	56.3 ± 10.5	57.3 ± 10.7	58.6 ± 11.1	60.0 ± 11.6	62.2 ± 12.7	<0.0001
Prepregnancy BMI (kg/m^2)	21.9 ± 3.9	22.3 ± 4.0	22.6 ± 4.1	23.1 ± 4.3	23.8 ± 4.8	<0.0001
GWG (kg)	9.5 ± 4.5	10.0 ± 4.4	10.4 ± 4.5	10.8 ± 4.8	11.4 ± 5.1	<0.0001
GWG (100 g) per gestational week	2.46 ± 1.14	2.59 ± 1.14	2.68 ± 1.15	2.80 ± 1.25	2.96 ± 1.32	<0.0001
			%			
Parity, nulliparous	34.8	32.8	30.1	28.3	24.3	<0.0001
Race						
White	40.7	44.3	46.1	48.2	51.5	<0.0001
Black	52.1	48.7	46.0	44.5	40.9	
Other	7.2	7.1	7.9	7.3	7.7	
Education						
<High school	28.0	26.9	27.8	27.1	28.5	0.19
≥High school	72.0	73.1	72.2	72.9	71.5	
Smoke during pregnancy (cigarettes per day)						
No	52.7	52.5	53.0	52.1	53.4	0.0001
<10	28.7	28.2	27.3	27.6	25.4	
11-20	15.1	15.5	15.8	16.1	16.3	
>20	3.5	3.7	3.9	4.2	4.8	
Infant factors						
Sex, male	50.9	51.0	50.7	50.2	51.1	0.85
Gestational age (weeks)	38.7 ± 2.5	38.7 ± 2.5	38.7 ± 2.5	38.7 ± 2.5	38.7 ± 2.5	0.99
Preterm	15.3	15.0	15.6	14.9	15.3	0.81
Birth weight (g)	2779 ± 481	3030 ± 430	3169 ± 424	3322 ± 431	3584 ± 476	<0.0001
Birth weight-for-gestational age *z*-score	−0.99 ± 0.86	−0.46 ± 0.78	−0.17 ± 0.79	0.15 ± 0.83	0.71 ± 0.94	<0.0001

GWG: gestational weight gain. χ^2 test for categorical variables and ANOVA *F*-test for continuous variables.

(1,28). We used 2000 Centers for Disease Control and Prevention (CDC) growth charts as the reference 29.

Covariates

Maternal sociodemographic factors (age, race/ethnicity, and education), smoking and parity were obtained during the initial prenatal interview using standardized questionnaires. Gestational age at birth was calculated from the last menstrual period (LMP). Birth weight was obtained at delivery by study nurses. We calculated birth weight-for-gestational age *z*-score according to internal mean and SD of birth weight in the stratum of corresponding ethnic group, sex, and gestational week in singleton births to nonsmoker mothers in the CPP cohort. Also, based on internal race- and sex-specific references at each gestational week by the global reference weight percentile calculator (30), we defined infant small for gestational age (SGA), appropriate for gestational age (AGA), and LGA, respectively, as birth weight <10th, 10th-90th, and >90th percentile.

Statistical analysis

First we compared maternal and infant characteristics and prenatal factors (prepregnancy obesity, GWG, and GDM) across quintiles of placental weight (Tables 1 and 2), using ANOVA *F*-test for continuous variables and χ^2 tests for categorical data. Next, we used locally weighted nonparametric smoothing scatter plots (SAS LOESS, SAS Institute, Cary, NC) to graphically examine the relationship of sex-

TABLE 2 Prenatal and perinatal factors of study participants by gestational age- and sex-specific quintiles of placental weight

	Placental weight (g)	Placental weight *z*-score[a]	Placental weight quintile Q1 (*N* = 6746)	Q2 (*N* = 6836)	Q3 (*N* = 6778)	Q4 (*N* = 6803)	Q5 (*N* = 6730)	*P* value
Prepregnancy BMI (kg/m^2)	Mean (SD)				%			
<18.5	408 ± 87	−0.29 ± 0.92	13.4	10.8	9.3	7.3	5.8	<0.0001
18.5-24.9	433 ± 92	−0.04 ± 0.97	70.8	71.2	70.1	69.0	63.8	
25-29.5	456 ± 97	0.21 ± 1.04	11.5	13.2	14.9	16.6	20.5	
≥30	470 ± 105	0.35 ± 1.13	4.3	4.8	5.8	7.1	9.9	
GWG per 2009 IOM guidelines								
Inadequate	420 ± 88	−0.17 ± 0.94	59.6	54.5	49.8	44.8	36.2	<0.0001
Adequate	445 ± 94	0.08 ± 1.00	29.5	32.3	33.7	35.3	37.6	
Excessive	464 ± 102	0.32 ± 1.07	10.9	13.2	16.5	19.9	26.2	
GDM								
No	436 ± 94	−0.01 ± 0.99	99.3	99.6	99.1	99.0	97.8	<0.0001
Yes	488 ± 121	0.65 ± 1.29	0.7	0.4	0.9	1.0	2.2	
Infant birth weight for gestational age								
SGA	362 ± 73	−0.86 ± 0.75	37.2	15.2	9.2	4.6	1.5	
AGA	436 ± 84	−0.03 ± 0.87	58.3	75.8	76.5	73.1	57.3	<0.0001
LGA	490 ± 107	0.74 ±1.06	4.5	9.0	14.3	22.3	41.2	

AGA, appropriate for gestational age; GWG, gestational weight gain; GDM, gestational diabetes mellitus; LGA, large for gestational age; SGA: small for gestational age.
χ^2 test for categorical variables.
[a]Sex- and gestational age-specific *z*-score of placental weight.

and gestational age-specific placental weight quintiles with postnatal BMI across childhood ages with stratification by preterm status. Since the patterns were similar in term and preterm births, we presented the pooled results (Figure 1). We used generalized estimating equation (GEE)-linear and logistic regression to evaluate the relationship of prenatal factors (prepregnancy BMI, GWG, GDM) and placental weight (expressed on both continuous and categorical scale) with childhood BMI (Tables 3 and 4) and obesity (Table 5).

We performed sequential models to evaluate a possible mediation effect of placental weight on prenatal factors (X) in relation to childhood BMI (31). First, we presented a univariate model (Model 0). Then, we adjusted for covariates of *a priori* interest including demographics, maternal smoking, preterm status, and parity—factors known to influence childhood obesity (Model 1: childhood BMI = $i_1 + cX +$ covariates + e_1) (32). Next, we added placental weight in the models to examine the extent to which placental weight mediates the relationship between each prenatal factor and childhood weight outcomes (Model 2: childhood BMI = $i_2 + b$·placental weight *z*-score + $c'X$ + covariates + e_2). For our models examining associations of GDM and GWG with childhood weight outcomes, we additionally adjusted for prepregnancy BMI categories, as it may confound

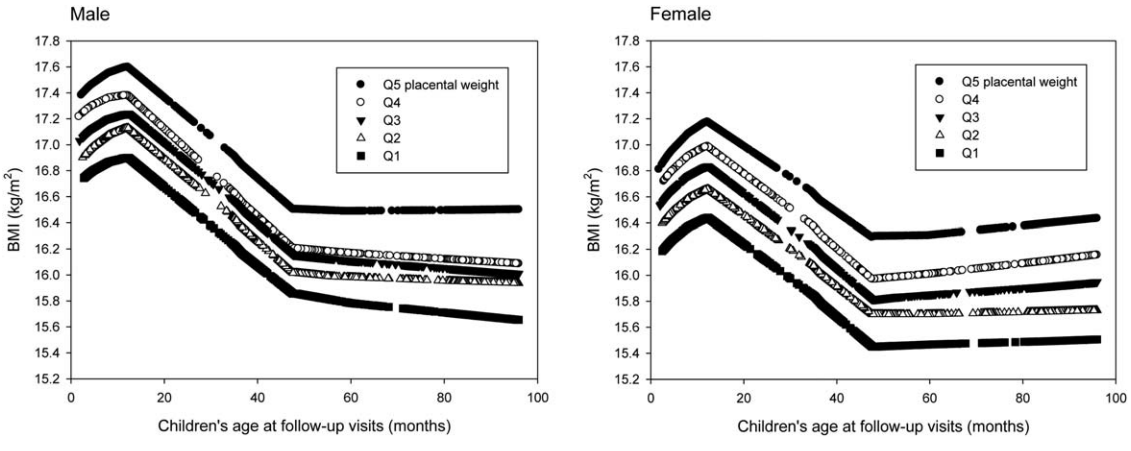

Figure 1 Smoothing scatter plots of children's BMI by age, stratified by gestational age- and sex-specific quintiles (Q1-Q5) of placental weight.

TABLE 3 Association of prenatal factors and placental weight with BMI in children

Prenatal and perinatal predictor	Model 0: crude model	Model 1: each predictor + covariates[a]	Model 2: Model 1 + placental weight *z*-score[b]	Model 3: Model 1 + prepregnancy BMI categories[c]	Model 4: Model 3 + placental weight *z*-score[b]	Mediation effect by placental weight in the associations between each predictor and childhood BMI (95% CI)
	Increment in children's BMI (kg/m^2) β (95% CI) for 1-unit increment in predictor					
Prepregnancy BMI (kg/m^2)	0.05 (0.05, 0.06)	0.06 (0.06, 0.07)	0.05 (0.05, 0.06)	NA	NA	0.008 (0.007, 0.009)
GWG (100 g/week)	0.09 (0.08, 0.10)	0.10 (0.09, 0.11)	0.07 (0.05, 0.08)	0.12 (0.11, 0.13)	0.09 (0.08, 0.10)	0.02 (0.02, 0.03)
GDM (yes vs. no)	0.43 (0.25, 0.60)	0.29 (0.11, 0.46)	0.15 (−0.02, 0.32)	0.23 (0.05, 0.40)	0.11 (−0.06, 0.28)	0.14 (0.11, 0.18)
Placental weight *z*-score[b]	0.26 (0.25, 0.28)	0.25 (0.23, 0.26)	NA	0.22 (0.20, 0.23)	NA	NA

GWG, gestational weight gain; GDM, gestational diabetes mellitus; NA: not available.
[a]Covariates included maternal age, education, race, smoke during pregnancy, parity (nulliparous vs. others), preterm status, child's sex, age, age^2, and hospital site.
[b]Placental weight *z*-score: sex- and gestational age-specific placental weight *z*-score.
[c]Prepregnancy BMI categories: underweight, normal weight, overweight, and obesity.

these associations (i.e., Model 3: childhood BMI = $i_3 + cX$ + covariates + prepregnancy BMI categories + e_3) (33) and then evaluated the mediation effect of placental weight (Model 4: childhood BMI = $i_4 + b$ placental weight *z*-score + $c'X$ + covariates + prepregnancy BMI categories + e_4). As a mediator, placental weight should predict outcome in a model controlling for predictors of interest (i.e., prenatal factors). We also calculated the mediated effect of placental weight (in *z*-score) = $a{\cdot}b$ (31). The "a" is the parameter relating the prenatal factors (prepregnancy BMI, GWG, GDM) to the mediating variable (i.e., placental weight) in the model: placental weight *z*-score= $i_5 + a{\cdot}X + e_5$; b is the parameter relating mediators to the outcome variable with adjustment for the predictor variable (X) (Models 2 and 4) (31). 95% confidence interval (CI) of the mediated effect = $a{\cdot}b \pm 1.96s$, where $s = \sqrt{a^2 s_b^2 + b^2 s_a^2}$, and s_a and s_b are standard errors of a and b, respectively.

Finally, to assess whether there is a direct pathway from placental weight to childhood weight/BMI, which does not go through birth weight, we performed a Grand Model (with all predictors, covariates, and placental ovality) with and without birth weight status included in the models.

Because irregular placental shapes were associated with lower birth weight (12), we also conducted sensitivity analyses of the above GEE-linear regression models among those mother-infant pairs with the round/oval shaped placentas ($n = 32{,}506$ mother-infant pairs with 148,154 postnatal follow-up measures of childhood BMI).

We applied GEE to adjust for correlations among measures in multiple follow-up visits from the same child. All analyses were performed with SAS 9.1 software (SAS Institute).

The CPP data are publicly available through the U.S. National Archives (www.archives.gov). Research ethics approval was waived by the Institutional Review Board of Shanghai Xinhua Hospital because the study was based on publicly available de-identified dataset.

Results

Study population

Among 33,893 mothers, 15.3% were overweight, and 6.4% had obesity before pregnancy. Mean GWG was 10.4 kg (SD 4.7); 5875 (17.3%) had excessive GWG, and 350 (1%) had diagnosed GDM. From infancy to 7 years of age (total 154,590 follow-up visits), 7.2% had obesity. At the last follow-up visit (mean age, 6.3 years; SD: 2.1 years), 6.4% (2176/33893) of children had obesity with a mean (SD) BMI of 16.2 (2.0) kg/m^2.

The characteristics of 33,893 mother-infant pairs by placental weight quintile are presented in Table 1. Mothers with greater placental weight (from Q1 to Q5) were slightly older (mean age from 23.6 to 25.1 years) and more likely to be White (40.7% to 51.5%), but did not differ in education level ($P = 0.19$). For parity, there were more nulliparous mothers in the Q1 (lowest) placental weight group (34.8%) and less in the Q5 group (24.3%). Mean placental weight was higher in male versus female infants and lower for preterm versus term births (Supporting Information Table S1).

TABLE 4 Association of prenatal factors and placental weight with BMI in children, with and without adjustment for birth weight-for-gestational age *z*-score

	Grand Model 1: all predictors+ placental weight + covariates + placental ovality[a]	Grand Model 2: Grand Model 1 + birth weight-for-gestational age *z*-score
Prenatal and perinatal predictor	Increment in children's BMI (kg/m^2) β (95% CI) for 1-unit increment in predictor	
Prepregnancy BMI (kg/m^2)	0.06 (0.05, 0.06)	0.05 (0.05, 0.06)
GWG (100 g/week)	0.10 (0.08, 0.11)	0.06 (0.05, 0.07)
GDM (yes vs. no)	0.07 (−0.10, 0.24)	0.06 (−0.11, 0.23)
Placental weight *z*-score[b]	0.19 (0.18, 0.21)	0.04 (0.03, 0.06)

GWG, gestational weight gain; GDM, gestational diabetes mellitus.
Grand Model 1: All predictor variables (prepregnancy BMI + GWG + presence of GDM) + placental weight *z*-score + placental ovality + maternal age, education, race, smoking during pregnancy, parity (nulliparous vs. others), preterm status, child's sex, age, age^2, and hospital site.
[a]Placental ovality was assessed by the difference between the largest and smallest diameter (cm).
[b]Placental weight *z*-score: sex- and gestational age-specific placental weight *z*-score.

Maternal prenatal factors and placental weight

In women with prepregnancy obesity, excessive GWG, or GDM, placental weights were heavier with greater proportions in higher quintiles of placental weight (Table 2). For example, mean placental weight was 470 versus 433 g for women with prepregnancy obesity versus normal weight. The proportion of prepregnancy obesity was 4.3% in Q1 and 9.9% in Q5 group. Similarly, more infants were LGA from placental weight Q1 (4.5%) to Q5 (41.2%) groups (Table 2).

Placental weight and offspring BMI during infancy and childhood

Smoothing plots showed that children in the highest quintile of placental weight had consistently the highest BMIs across all ages in both sexes (Figure 1). There were clearly increasing childhood BMIs from the lowest to highest quintiles of placental weight.

The extent to which placental weight may mediate prenatal factors and childhood BMI/risk of obesity

With each 1 kg/m^2 increase in maternal prepregnancy BMI, placental weight *z*-score was 0.04 unit higher (95% CI: 0.04, 0.04, SE = 0.001, Supporting Information Table S2), and childhood BMI was higher by 0.06 kg/m^2 (95% CI: 0.06, 0.07, Table 3 Model 1) with adjustment for maternal age, education, race, smoking during pregnancy, parity, preterm status, child's age and sex, and hospital site. When we entered placental weight *z*-score into the model of the association between maternal BMI and childhood BMI, the effect estimate for maternal BMI was attenuated by 17% (from 0.06 to 0.05, Table 3, Models 1 and 2). The mediated effect through placental weight was 0.008 (95% CI: 0.008, 0.009) kg/m^2 of children's BMI for each kg/m^2 increment in prepregnancy BMI (Table 3).

On average, each 100 g GWG per week was associated with 0.12 (95% CI: 0.11, 0.13) kg/m^2 higher childhood BMI (Table 3, Model 3). Adding placental weight to the models attenuated the effect estimates on childhood BMI by 25% for weekly GWG (Table 3, Models 3 and 4). The mediated effect through placental weight was 0.02 (95% CI: 0.02, 0.03) kg/m^2 of children's BMI for each 100 g of weekly GWG (Table 3 and Supporting Information Table S2).

BMI was 0.23 (95% CI: 0.05, 0.40) kg/m^2 higher for children born to mothers with GDM versus without GDM (Table 3, Model 3s). Adding placental weight to the models attenuated the effect estimates on childhood BMI by 52% (Table 3, Models 3 and 4). The mediated effect of GDM through placental weight was 0.14 (95% CI: 0.11, 0.18) kg/m^2 of childhood BMI.

Placental weight *z*-score was consistently associated with higher childhood BMI ($\beta = 0.19$ kg/m^2, 95% CI: 0.18, 0.21) after adjusting for maternal obesity, excessive GWG, GDM, other covariates, and placental ovality (Table 4 Model 1). With additional adjustment for infant birth weight *z*-score, the effect estimate of placental weight was attenuated to 0.04 (0.03, 0.06) but still significant ($P < 0.05$) (Table 4). This suggests that there might be another direct pathway between placental weight and child weight, in addition to the pathway through birth weight.

The odds of childhood obesity were 1.95-fold (95% CI: 1.77, 2.15) higher for children born to mothers having obesity versus normal weight after adjustment for covariates, and the OR was attenuated to 1.82 (95% CI: 1.65, 2.00) after adding placental weight to the models (Table 5, Models 1 and 2).

The odds of childhood obesity were 1.15-fold (1.07, 1.24) higher for excessive versus adequate GWG and 1.45-fold (1.17, 1.81) higher for children born mothers with GDM versus without GDM (Table 5, Model 3); adding placental weight to the models attenuated these effect estimates of OR to 1.11 (1.03, 1.20) and 1.33 (1.07, 1.65), respectively (Table 5, Model 4).

Also, with additional adjustment for birth weight-for-gestational age status (LGA, AGA, and SGA), the odds of childhood obesity were 1.46-fold higher for Q5 versus Q1 placental weight (95% CI: 1.33, 1.60) (test of trend $P < 0.001$, Table 5 Model 5).

If the analyses were restricted to mother-child pairs with oval/round shaped placenta, we found similar results on mediation effect of

TABLE 5 Association of prenatal factors and placental weight with the prevalence of obesity in children

		Obesity					
	n (%)[a]	Model 0: crude model	Model 1: each predictor + covariates[b]	Model 2: Model 1 + placental weight quintiles	Model 3: Model 1 + prepregnancy BMI categories	Model 4: Model 3 + placental weight quintiles	Model 5: all predictors + covariates + placental ovality[c]
		OR (95% CI)					
Prepregnancy BMI (kg/m^2)							
<18.5	591 (4.2)	0.61 (0.55, 0.68)	0.61 (0.55, 0.69)	0.65 (0.58, 0.72)			0.67 (0.60, 0.75)
18.5-24.9	7066 (6.7)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	NA	NA	1.00 (Ref.)
25-29.5	2236 (9.3)	1.44 (1.35, 1.55)	1.46 (1.36, 1.56)	1.39 (1.29, 1.49)			1.29 (1.20, 1.39)
≥30	1210 (11.8)	1.87 (1.70, 2.05)	1.95 (1.77, 2.15)	1.82 (1.65, 2.00)			1.67 (1.51, 1.84)
GWG							
Inadequate	4501 (6.0)	0.77 (0.72, 0.81)	0.75 (0.71, 0.80)	0.80 (0.75, 0.85)	0.79 (0.75, 0.84)	0.83 (0.78, 0.88)	0.85 (0.80, 0.90)
Adequate	4013 (7.7)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Excessive	2589 (9.5)	1.25 (1.16, 1.34)	1.30 (1.21, 1.40)	1.23 (1.15, 1.33)	1.15 (1.07, 1.24)	1.11 (1.03, 1.20)	1.09 (1.01, 1.17)
GDM							
No	10,905 (7.1)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
Yes	198 (12.5)	1.84 (1.49, 2.28)	1.55 (1.25, 1.93)	1.39 (1.12, 1.73)	1.45 (1.17, 1.81)	1.33 (1.07, 1.65)	1.32 (1.06, 1.64)
Placental weight							
Q1	1604 (5.1)	1.00 (Ref.)	1.00 (Ref.)	NA	1.00 (Ref.)	NA	1.00 (Ref.)
Q2	1905 (6.1)	1.19 (1.08, 1.30)	1.17 (1.07, 1.29)		1.15 (1.05, 1.26)		1.06 (0.96, 1.16)
Q3	2076 (6.7)	1.34 (1.22, 1.46)	1.32 (1.21, 1.44)		1.27 (1.16, 1.39)		1.11 (1.01, 1.22)
Q4	2381 (7.7)	1.57 (1.43, 1.71)	1.56 (1.42, 1.70)		1.47 (1.34, 1.60)		1.23 (1.12, 1.35)
Q5	3137 (10.4)	2.13 (1.96, 2.32)	2.06 (1.89, 2.25)		1.89 (1.73, 2.06)		1.46 (1.33,1.60)
Birth weight for gestational age							
SGA	995 (4.8)	0.66 (0.60, 0.72)	0.62 (0.57, 0.68)	0.71 (0.65, 0.79)	0.65 (0.59, 0.71)	0.72 (0.66, 0.80)	0.73 (0.67, 0.81)
AGA	7388 (7.0)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)	1.00 (Ref.)
LGA	2720 (9.7)	1.42 (1.33, 1.51)	1.67 (1.56, 1.80)	1.43 (1.32, 1.54)	1.58 (1.47, 1.70)	1.38 (1.28, 1.49)	1.34 (1.24, 1.45)

AGA, appropriate for gestational age; GWG, gestational weight gain; GDM, gestational diabetes mellitus; NA, not available; LGA, large for gestational age; SGA, small for gestational age.
[a]The number and % of obesity among children's postnatal follow-up measures.
[b]Covariates included maternal age, maternal education, maternal race, parity (nulliparous vs. others), smoking during pregnancy (yes/no), preterm status, child sex, age, age^2, and hospital of delivery.
[c]Placental ovality was assessed by the difference between the largest and smallest diameter (cm).

placental weight in the associations between the three prenatal factors and childhood BMI (Supporting Information Table S3).

Discussion

In this prospective birth cohort study, we found that prepregnancy obesity, excessive GWG, and GDM were all associated with greater childhood BMI and higher risk of childhood obesity from infancy to 7 years of age. Inclusion of placental weight in models attenuated these associations, suggesting that placental weight may partly mediate the effects of these prenatal factors. We also found that higher placental weight was an independent predictor of offspring BMI and obesity.

There is growing evidence that the placenta plays a key role in fetal programming of cardiometabolic disease (21). However, only one previous study of about 800 children examined the associations of placental weight with childhood weight status at 6 months, and in contrast to our study, did not find association between placental weight and infant weight (17). The analyses from this previous study were adjusted for birth weight (17).

We previously found that placental weight at birth was associated with birth weight for gestational age and partially mediated the associations between prenatal risk factors and LGA (15). In this large-scale prospective cohort study, we extended those findings to childhood weight outcomes from infancy to 7 years of age. Our study indicates that the placenta may contribute to the *in utero* programming of childhood obesity.

Our findings on the partial mediation effect of placental weight between these prenatal factors and offspring obesity are biologically plausible. Placental size is correlated with capacity for nutrient transfer and increases steadily throughout gestation (34). Both

animal models and human studies indicate that nourishment prior to pregnancy and hyperglycemia during early pregnancy increase placental growth which results in increased placental nutrient transport activity (35,36). Prepregnancy obesity and excessive GWG both were risk factors of GDM (13,14). Increased nutrient or glucose availability in obese and GDM pregnancy affects placental growth and therefore placental transport efficiency (36). In a study of 300 mother-infant pairs from the CPP, placental weight was found to be a better surrogate for maternal biomarker (estrogen) level during pregnancy than birth weight (37). While reduced placental growth leads to restricted fetal growth, high placental weight is correlated with higher prevalence of LGA (16,17). In this study, even with adjustment for birth weight status, placental weight was associated with childhood obesity. We speculate that a larger placenta and an *in utero* over-nutrition environment may impact future offspring metabolism and growth and program the susceptibility to obesity in children.

Due in part to the association between placental size and risk of obesity in children, we found a substantial mediation effect of placental weight on the associations of prepregnancy BMI, weekly GWG, and GDM with childhood BMI (17-52%). For the part not explained by placental weight, it could be due to genetic susceptibility (e.g., prepregnancy obesity), family eating environment (for example, parent-child similarities) and physical activity habit, the intrauterine hormonal milieu, or other yet unknown pathways (38,39).

In this study, we observed a linear trend of placental weight with childhood BMI/obesity, instead of "U" shaped associations. It is noticeable that BMI can reflect both lean body mass and body fat.

Strengths of this study include the large sample size and prospective nature of birth cohort design. The prospective design observed the temporal order of all the events and minimized observer information bias. In addition, carefully measured placental data, multiple prenatal and perinatal data, and follow-up anthropometric measures throughout childhood provided a unique opportunity to test our hypothesis. Also, since the CPP was conducted before the obesogenic environment emerged, the results were less likely to be confounded by the obesogenic factors.

Several limitations are worth mentioning. First, the CPP was conducted 50 years ago. The relevance of our findings to the contemporary society may be questioned because of lower prevalence of maternal and offspring obesity and GDM in this study. Nevertheless, the effect estimates of prepregnancy obesity on childhood obesity (OR = 1.49 and 1.95) were comparable to the findings in other recent studies (6). Second, in the era that data was collected in the CPP, the OGTT cutoff points for GDM diagnosis were higher than the recent or current criteria (40). In addition, GDM was not routinely screened, and standardized criteria for its diagnosis were not routinely followed. Thus, GDM may have been "under-diagnosed," a misclassification that may bias our findings toward the null, if any. Third, gestational age was estimated based on self-reported LMP and recall bias may exist. Nevertheless, the data were collected at the initial prenatal care visit, and the women did not know their pregnancy outcomes yet; thus recall bias, if any, was non-differential. Finally, although placental weight is an important parameter, it is not equivalent to placental function for nutrient transfer or endocrine function. Our epidemiologic findings provide the basis for future studies on the role of placenta in mediating the effect of prenatal factors on childhood obesity.

Conclusion

In this U.S. large-scale multicenter birth cohort, placental weight was an independent predictor of childhood obesity and in part mediated the associations of maternal weight status and GDM with childhood obesity. Our study suggests long-term effect of the placenta on the risk of childhood obesity.O

References

1. Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007-2008. *JAMA* 2010;303:242-249.
2. Baker JL, Olsen LW, Sorensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. *N Engl J Med* 2007;357:2329-2337.
3. Chu SY, Kim SY, Bish CL. Prepregnancy obesity prevalence in the United States, 2004-2005. *Matern Child Health J* 2009;13:614-620.
4. Bardenheier BH, Imperatore G, Gilboa SM, et al. Trends in gestational diabetes among hospital deliveries in 19 U.S. states, 2000-2010. *Am J Prev Med* 2015;49:12-19.
5. Oken E, Rifas-Shiman SL, Field AE, Frazier AL, Gillman MW. Maternal gestational weight gain and offspring weight in adolescence. *Obstet Gynecol* 2008;112:999-1006.
6. Mesman I, Roseboom TJ, Bonsel GJ, Gemke RJ, van der Wal MF, Vrijkotte TG. Maternal pre-pregnancy body mass index explains infant's weight and BMI at 14 months: Results from a multi-ethnic birth cohort study. *Arch Dis Child* 2009;94:587-595.
7. Ludwig DS, Currie J. The association between pregnancy weight gain and birthweight: a within-family comparison. *Lancet* 2010;376:984-990.
8. Oken E, Taveras EM, Kleinman KP, Rich-Edwards JW, Gillman MW. Gestational weight gain and child adiposity at age 3 years. *Am J Obstet Gynecol* 2007;196:322 e1-328.
9. Schaefer-Graf UM, Heuer R, Kilavuz O, Pandura A, Henrich W, Vetter K. Maternal obesity not maternal glucose values correlates best with high rates of fetal macrosomia in pregnancies complicated by gestational diabetes. *J Perinat Med* 2002;30:313-321.
10. Gillman MW, Rifas-Shiman S, Berkey CS, Field AE, Colditz GA. Maternal gestational diabetes, birth weight, and adolescent obesity. *Pediatrics* 2003;111:e221-e226.
11. Lisboa PC, de Oliveira E, de Moura EG. Obesity and endocrine dysfunction programmed by maternal smoking in pregnancy and lactation. *Front Physiol* 2012;3:437):437(
12. Yampolsky M, Salafia CM, Shlakhter O, Haas D, Eucker B, Thorp J. Modeling the variability of shapes of a human placenta. *Placenta* 2008;29:790-797.
13. Abenhaim HA, Kinch RA, Morin L, Benjamin A, Usher R. Effect of prepregnancy body mass index categories on obstetrical and neonatal outcomes. *Arch Gynecol Obstet* 2007;275:39-43.
14. Hillier TA, Pedula KL, Vesco KK, et al. Excess gestational weight gain: modifying fetal macrosomia risk associated with maternal glucose. *Obstet Gynecol* 2008;112:1007-1014.
15. Ouyang F, Parker M, Cerda S, et al. Placental weight mediates the effects of prenatal factors on fetal growth: the extent differs by preterm status. *Obesity (Silver Spring)* 2013;21:609-620.
16. L'Abee C, Vrieze I, Kluck T, Erwich JJ, Stolk RP, Sauer PJ. Parental factors affecting the weights of the placenta and the offspring. *J Perinat Med* 2011;39:27-34.
17. Hindmarsh PC, Geary MP, Rodeck CH, Kingdom JC, Cole TJ. Factors predicting ante- and postnatal growth. *Pediatr Res* 2008;63:99-102.
18. Taricco E, Radaelli T, Nobile de Santis MS, Cetin I. Foetal and placental weights in relation to maternal characteristics in gestational diabetes. *Placenta* 2003;24:343-347.
19. Constancia M, Hemberger M, Hughes J, et al. Placental-specific IGF-II is a major modulator of placental and fetal growth. *Nature* 2002;417:945-948.
20. Mayhew TM, Ohadike C, Baker PN, Crocker IP, Mitchell C, Ong SS. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. *Placenta* 2003;24:219-226.
21. Thornburg KL, O'Tierney PF, Louey S. Review: the placenta is a programming agent for cardiovascular disease. *Placenta* 2010;31 (Suppl):S54-S59.
22. Hardy JB. The Collaborative Perinatal Project: lessons and legacy. *Ann Epidemiol* 2003;13:303-311.
23. Klebanoff MA. The Collaborative Perinatal Project: A 50-year retrospective. *Paediatr Perinat Epidemiol* 2009;23:2-8.

24. Institute of Medicine. *Weight Gain During Pregnancy: Reexamining the Guidelines*. Washington, DC: National Academies Press; 2009.
25. Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. National Diabetes Data Group. *Diabetes* 1979;28:1039-1057.
26. Baptiste-Roberts K, Nicholson WK, Wang NY, Brancati FL. Gestational diabetes and subsequent growth patterns of offspring: the National Collaborative Perinatal Project. *Matern Child Health J* 2012;16:125-132.
27. Winder NR, Krishnaveni GV, Veena SR, et al. Mother's lifetime nutrition and the size, shape and efficiency of the placenta. *Placenta* 2011;32:806-810.
28. Krebs NF, Himes JH, Jacobson D, Nicklas TA, Guilday P, Styne D. Assessment of child and adolescent overweight and obesity. *Pediatrics* 2007;120 (Suppl 4):S193-S228.
29. CDC. Avavilable at: http://www.cdc.gov/nccdphp/dnpao/growthcharts/resources/sas.htm. Accessed August 14th, 2010.
30. Mikolajczyk RT, Zhang J, Betran AP, et al. A global reference for fetal-weight and birthweight percentiles. *Lancet* 2011;377:1855-1861.
31. MacKinnon DP. Introduction to Statistical Mediation Analysis. New York, NY: Lawrence Erlbaum Associates, Taylor & Francis Group; 2008.
32. Steyn K, de Wet T, Saloojee Y, Nel H, Yach D. The influence of maternal cigarette smoking, snuff use and passive smoking on pregnancy outcomes: the Birth To Ten Study. *Paediatr Perinat Epidemiol* 2006;20:90-99.
33. Salafia CM, Zhang J, Charles AK, et al. Placental characteristics and birthweight. *Paediatr Perinat Epidemiol* 2008;22:229-239.
34. Fowden AL, Ward JW, Wooding FP, Forhead AJ, Constancia M. Programming placental nutrient transport capacity. *J Physiol* 2006;572:5-15.
35. Ericsson A, Saljo K, Sjostrand E, et al. Brief hyperglycaemia in the early pregnant rat increases fetal weight at term by stimulating placental growth and affecting placental nutrient transport. *J Physiol* 2007;581:1323-1332.
36. Higgins L, Greenwood SL, Wareing M, Sibley CP, Mills TA. Obesity and the placenta: a consideration of nutrient exchange mechanisms in relation to aberrant fetal growth. *Placenta* 2011;32:1-7.
37. Teasdale F, Jean-Jacques G. Morphometry of the microvillous membrane of the human placenta in maternal diabetes mellitus. *Placenta* 1986;7:81-88.
38. Longtine MS, Nelson DM. Placental dysfunction and fetal programming: the importance of placental size, shape, histopathology, and molecular composition. *Semin Reprod Med* 2011;29:187-196.
39. Cutting TM, Fisher JO, Grimm-Thomas K, Birch LL. Like mother, like daughter: familial patterns of overweight are mediated by mothers' dietary disinhibition. *Am J Clin Nutr* 1999;69:608-613.
40. ADA. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2013;36 (Suppl 1):S67-S74.