

Roles of Birth Mode and Infant Gut Microbiota in Intergenerational Transmission of Overweight and Obesity From Mother to Offspring

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IMPORTANCE Maternal overweight, which often results in cesarean delivery, is a strong risk factor for child overweight. Little is known about the joint contribution of birth mode and microbiota in the infant gut to the association between maternal prepregnancy overweight and child overweight.

OBJECTIVE To investigate the association of birth mode with microbiota in the infant gut, and whether this mediates the association between maternal and child overweight.

DESIGN, SETTING, AND PARTICIPANTS An observational study was conducted of 935 full-term infants born between January 1, 2009, and December 31, 2012, in the Canadian Healthy Infant Longitudinal Development (CHILD) birth cohort. Maternal prepregnancy body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared using height and weight data taken from medical records or maternal report. Infant gut microbiota were profiled with 16S ribosomal RNA sequencing in fecal samples collected at a mean (SD) age of 3.7 (1.0) months. At ages 1 and 3 years, BMI z scores adjusted for age and sex were generated according to World Health Organization criteria. Statistical analysis was conducted from January 29 to June 15, 2017.

EXPOSURES Mothers of normal weight (BMI, 18.5-24.9) and overweight or obese (BMI, ≥ 25.0) mothers.

MAIN OUTCOME AND MEASURES Risk of overweight and obesity (>97th percentile BMI z scores) among children at ages 1 and 3 years.

RESULTS Of the 935 mother-infant pairs in the study (mean [SD] age, 32.5 [4.5] years) 382 (40.9%) were overweight, 69 of 926 infants (7.5%) were overweight at age 1 year, and 90 of 866 infants (10.4%) were overweight at age 3 years. Compared with being born vaginally to a mother of normal weight, infants born vaginally to overweight or obese mothers were 3 times more likely to become overweight at age 1 year (adjusted odds ratio [OR], 3.33; 95% CI, 1.49-7.41), while cesarean-delivered infants of overweight mothers had a 5-fold risk of overweight at age 1 year (adjusted OR, 5.02; 95% CI, 2.04-12.38). Similar risks were also observed at age 3 years. Multiple mediator path modeling revealed that birth mode and infant gut microbiota (Firmicutes species richness, especially of the Lachnospiraceae family) sequentially mediated the association between maternal prepregnancy overweight and childhood overweight at ages 1 and 3 years. Bacterial genera belonging to the Lachnospiraceae family were more abundant in infants of overweight mothers; however, the participating genera of Lachnospiraceae differed between infants delivered vaginally and those delivered via cesarean birth.

CONCLUSIONS AND RELEVANCE This study found evidence of a novel sequential mediator pathway involving birth mode and Firmicutes species richness (especially higher abundance of Lachnospiraceae) for the intergenerational transmission of overweight.

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← Editorial page 320

+ Supplemental content

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Childhood obesity is a global health concern.^{1,2} More than 20% of preschool-aged children in Canada are overweight or obese (OWOB).³ Also on the rise is maternal OWOB during pregnancy⁴⁻⁶ and associated higher rates of birth by cesarean delivery.⁶⁻⁸ Furthermore, carrying OWOB into pregnancy presents, at minimum, a 2-fold greater risk of obesity in offspring.^{7,9,10} Some of this excess risk can be attributed to cesarean delivery, as children delivered by this method are 30% more likely to develop OWOB compared with those delivered vaginally.¹¹ However, while the epidemiologic evidence is strong that prenatal maternal obesity predisposes newborns to develop OWOB, the mechanism behind this intergenerational association has not, to our knowledge, been delineated by known genetic or lifestyle factors shared between mothers and their offspring.¹²

Mother-to-newborn transfer of obesogenic microbes has been put forward as a biological pathway for the intergenerational transmission of OWOB.¹³ Substantial changes to the intestinal microbiota of women are observed as pregnancy progresses to parturition.^{14,15} The weight status of women entering pregnancy can modify this process. Compared with their normal-weight counterparts, women with a high prepregnancy body mass index (BMI) have elevated levels of *Bacteroides* in their third trimester.¹⁶ Because maternal microbiota are the primary source for the first inoculation of newborn infants¹⁷ and microbiota have been implicated in adipogenesis,^{18,19} it is conceivable that prepregnancy weight and the maternal intestinal microbiome influence microbial assembly in the infant gut, as well as OWOB outcomes.

Several studies have investigated maternal OWOB-associated changes in microbiota of the infant gut.²⁰ Prepregnancy overweight has been found to affect microbial composition in infant stool, manifested as increases in *Bacteroides* species, at the ages of 1 month, 6 months,²¹ and 2 years.¹³ Microbial compositional changes associated with maternal OWOB are more evident in newborns after vaginal rather than cesarean delivery, and, for the latter, reductions in *Bacteroides* are seen.²² No gut dysbiosis in relation to maternal OWOB has been found in later infancy at ages 9 and 18 months.²³ Although cesarean delivery alters the structure of gut microbiota during early life,^{24,25} it remains to be determined whether birth mode plays a role in maternal OWOB-related changes to the microbiota of the infant gut and subsequent risk for OWOB. To address this gap in knowledge, we determined the joint associations of maternal prepregnancy OWOB and birth mode with microbial composition of the infant gut and microbiota interactions at ages 3 to 4 months, and with OWOB outcomes at ages 1 and 3 years. Second, we assessed whether microbiota of the infant gut mediated the association between maternal OWOB and child OWOB, and whether the mediation was dependent on birth mode.

Methods

Study Design

This study involved a subsample of 935 Canadian full-term infants from the Canadian Healthy Infant Longitudinal Devel-

Key Points

Question Do birth mode and microbiota in the infant gut mediate the association between maternal prepregnancy overweight and childhood overweight?

Findings In this cohort study of 935 mother-infant pairs, both vaginally and cesarean-delivered infants born to overweight and obese mothers were at greater risk of being overweight at ages 1 and 3 years compared with infants born vaginally to a mother of normal weight. Birth mode and microbiota in the infant gut (especially Lachnospiraceae) act sequentially to mediate the association between maternal prepregnancy overweight and child overweight.

Meaning Together, birth mode and Lachnospiraceae family microbiota in the infant gut sequentially mediate the intergenerational association of overweight, underscoring their potential contributions in developing child overweight and obesity.

opment (CHILD) birth cohort (<http://www.childstudy.ca>).²⁶ Mothers of studied infants were enrolled during pregnancy between January 1, 2009, and December 31, 2012. Maternal BMI was calculated as weight in kilograms divided by height in meters squared using measured height and self-reported prepregnancy weight or estimated from measured weight at 1 year post partum. Validation against prenatal records showed that prepregnancy weight was slightly underestimated by maternal recall (mean difference, -1.0 kg; 95% CI, -1.5 to -0.4 kg) and slightly overestimated by measured weight at 1 year post partum (mean difference, 1.3 kg; 95% CI, 0.5-2.2 kg). Maternal BMI was classified as normal weight (BMI, 18.5-24.9 [553 mothers]) and overweight and obese (BMI, ≥ 25.0 [382 mothers]). Child OWOB was defined as BMI z scores greater than the 97th percentile according to World Health Organization criteria²⁷; scores were generated from weight and height measured at ages 1 and 3 years. Data on covariates were obtained from hospital records (birth mode and intrapartum antibiotic prophylaxis [IAP]) or standardized questionnaires (maternal race/ethnicity, maternal asthma or allergy status, smoking during pregnancy, infant sex, infant direct antibiotic exposure by age 1 year, breastfeeding status before 3 months, sibblingship, and pet ownership). The Human Research Ethics boards at the University of Alberta, University of Manitoba, and University of British Columbia approved this study. Written informed consent was obtained from parents at enrollment.

Fecal Microbiota Analysis

Fecal samples of infants were collected at a mean (SD) age of 3.7 (1.0) months using a standard protocol during a planned home visit. Methods of sample collection, DNA extraction and amplification, 16S ribosomal RNA sequencing, and taxonomic classification have been previously described²⁸ (eAppendix in the Supplement).

Statistical Analysis

Statistical analysis was conducted from January 29 to June 15, 2017. The association between maternal OWOB and cesarean vs vaginal delivery, or a 4-category variable for birth mode

(vaginal without IAP, vaginal with IAP, scheduled cesarean delivery with IAP, and emergency cesarean delivery with IAP), was determined by binary and multinomial logistic regression, adjusting for location, infant sex, maternal race/ethnicity, maternal prenatal asthma, and maternal smoking during pregnancy. Logistic regression models were performed after covariate adjustment to test associations between maternal weight status (OWOB vs normal weight) or joint categories of maternal OWOB and birth mode with childhood OWOB (at ages 1 and 3 years) and microbiota of the infant gut (overall Chao1, Chao1, and Shannon indices of Firmicutes).

The association of maternal OWOB with microbiota of the infant gut (richness, α and β diversity, and relative abundance of taxa) was examined by a nonparametric Kruskal-Wallis test followed by post hoc comparison using the Dunn test. As evaluated by others,^{29,30} the coexistence of gut microbiota was measured as the Firmicutes to Bacteroidetes and Enterobacteriaceae to Bacteroidaceae ratios. Regression models of gut microbial measures (higher vs lower values based on a median cutoff) were performed against maternal OWOB and birth mode. Mediation analysis was conducted using the Hayes PROCESS macro in SPSS, version 23.0 (SPSS Inc), to permit evaluation of sequential mediators for the maternal-child OWOB association. A multiple mediator path model was evaluated to examine indirect associations of the 4-category variable of birth mode (mediator 1) and α diversity or relative abundance (categorized into tertiles) of microbiota of the infant gut at the family level (mediator 2). Bootstrapping, a nonparametric resampling procedure (5000 bootstrap resamples),^{31,32} was used to generate 95% CIs in mediation models. Microbial interaction networks by maternal-child OWOB group were built using the Spearman correlation coefficient ($\rho > 0.3$ or < -0.3) and visualized with Cytoscape, version 3.5.1.³³ To identify discriminative biomarkers for maternal OWOB, the linear discriminant analysis effect size (LEfSE) was determined with a linear discriminant analysis log score cutoff of 2.³⁴

Results

In this population-based cohort of 935 mother-infant pairs, 382 mothers (40.9%) were OWOB, 69 of 926 infants (7.5%) became OWOB at 1 year, and 90 of 866 infants (10.4%) became OWOB at 3 years (Table). Childhood weight status was associated with maternal weight status. Significant differences in the prevalence of childhood OWOB at age 1 or 3 years were found according to study location, infant sex, maternal prenatal asthma, maternal smoking during pregnancy, and direct exposure of infants to antibiotics. Prepregnancy rates of OWOB were higher in women who gave birth by cesarean delivery and who partially breastfed or did not breastfeed their infants.

Association Between Maternal OWOB and Cesarean Delivery

Almost half of mothers delivering by cesarean delivery had prepregnancy OWOB (Table). After covariate adjustment, maternal OWOB was associated with a 1.50 (95% CI, 1.10-2.07) times greater odds of cesarean delivery. A similar association

was also observed for maternal BMI z score (eFigure 1A in the Supplement). Overweight or obese mothers were more likely to undergo scheduled cesarean delivery or to have an emergency cesarean delivery than to deliver vaginally without IAP (eFigure 1B in the Supplement).

Impact of Maternal OWOB on Childhood OWOB at Ages 1 and 3 Years

As seen in Figure 1A and B, infants born to OWOB mothers were more likely to become OWOB at ages 1 year (adjusted odds ratio [OR], 3.80; 95% CI, 1.88-7.66) and 3 years (adjusted OR, 3.79; 95% CI, 2.10-6.84). This association was also significant with the maternal BMI z score (eTable 1 in the Supplement). Compared with vaginal delivery without IAP (reference group), emergency cesarean delivery was associated with a 2-fold greater odds of childhood OWOB at ages 1 and 3 years, but not after covariate adjustment (Figure 1A and B and eTable 1 in the Supplement). Joint associations of maternal weight status and birth mode with childhood OWOB were observed after covariate adjustment (Figure 1C and D). Compared with infants delivered vaginally to normal-weight mothers without IAP (reference group), vaginally delivered infants of OWOB mothers had approximately 3 times greater odds of OWOB at ages 1 year (3.33; 95% CI, 1.49-7.41) and 3 years (3.07; 95% CI, 1.58-5.96), whereas cesarean-delivered infants of OWOB mothers had approximately 5 times greater odds of OWOB at ages 1 year (5.02; 95% CI, 2.04-12.38) and 3 years (5.55; 95% CI, 2.55-12.04). The joint association with maternal OWOB was more prominent for emergency cesarean delivery than scheduled cesarean delivery (Figure 1C and D and eTable 1 in the Supplement).

Association of Maternal OWOB With Microbiota of the Infant Gut

Microbiota β diversity differed by maternal weight status (pseudo $F = 3.14$; $P = .001$ for unweighted UniFrac; pseudo $F = 2.45$; $P = .06$ for weighted UniFrac). Infants born to OWOB mothers had greater species richness (Chao1) in their gut microbiota, especially within the Firmicutes phyla; the diversity of the Firmicutes phyla was also higher (eTable 2 in the Supplement). Associations with higher Chao1 richness were independent of birth mode (eTable 3 in the Supplement). The Proteobacterial families Enterobacteriaceae and Pasteurellaceae were less abundant in infants born to OWOB vs normal-weight mothers, whereas 4 bacterial families (Coriobacteriaceae, Erysipelotrichaceae, Lachnospiraceae, and Ruminococcaceae) belonging to Actinobacteria and Firmicutes were more abundant (eTable 2 in the Supplement). Only maternal OWOB-associated changes in the Enterobacteriaceae to Bacteroidaceae ratio remained statistically significant after covariate adjustment (eTable 4 in the Supplement). Our LEfSe analysis indicated that several genera, including those of the Lachnospiraceae family, were significantly higher in infants born to OWOB mothers (eFigure 2 in the Supplement).

Joint Associations of Maternal OWOB and Birth Mode With Microbiota of the Infant Gut

Infants of OWOB mothers born via emergency cesarean delivery were twice more likely than vaginally born infants

Table. Population Characteristics and Their Associations With Maternal and Childhood OWOB

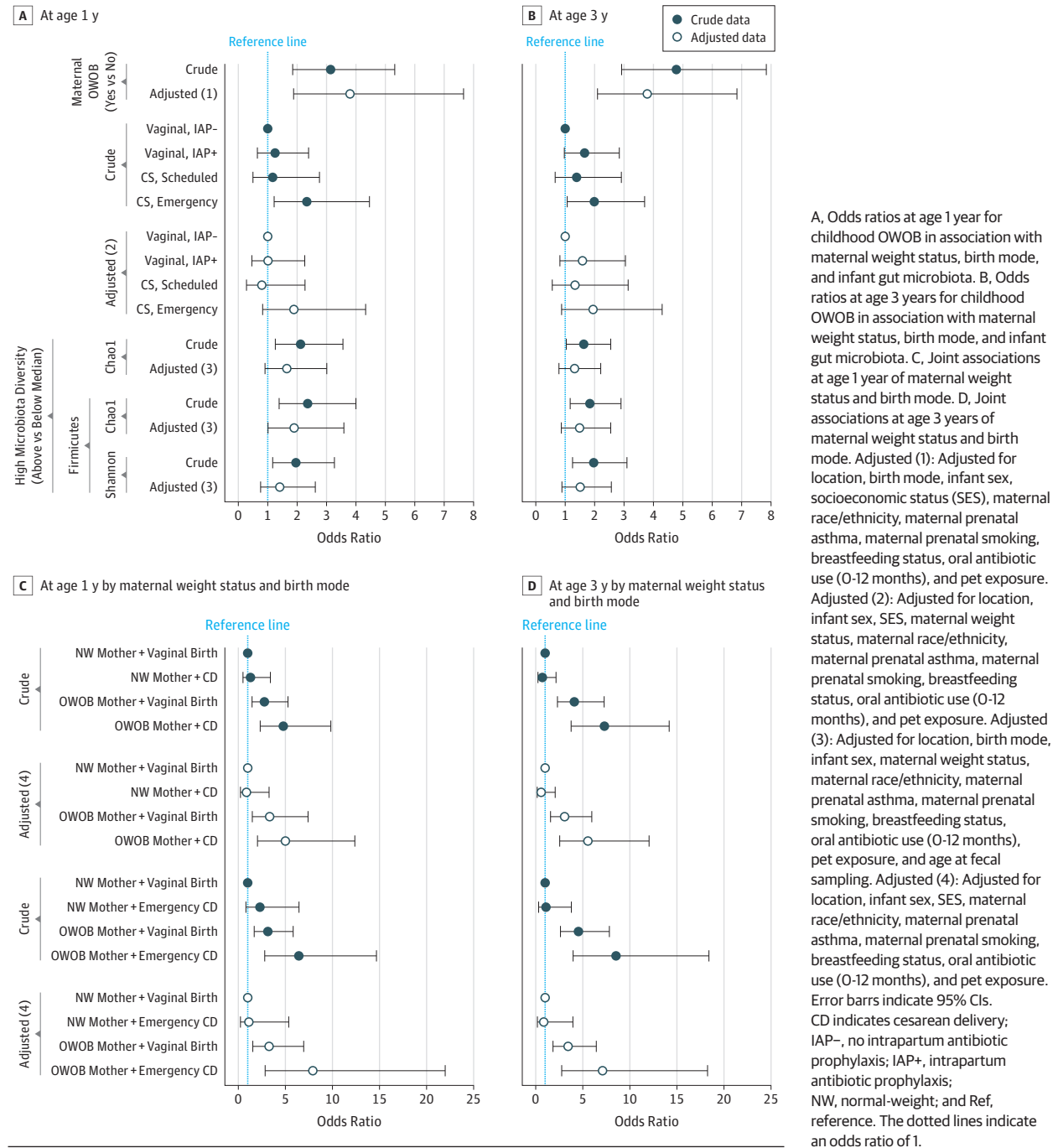
Characteristic	Maternal OWOB (n = 382/935 [40.9%])	P Value	Childhood OWOB at 1 y (n = 69/926 [7.5%])	P Value	Childhood OWOB at 3 y (n = 90/866 [10.4%])	P Value
Location, No. (%)						
Vancouver, British Columbia	94/323 (29.1)		21/326 (6.4)		16/304 (5.3)	
Edmonton, Alberta	150/324 (46.3)	<.001	31/323 (9.6)	.19	42/284 (14.8)	.001
Winnipeg, Manitoba	138/288 (47.9)		17/277 (6.1)		32/278(11.5)	
Birth mode, No. (%)						
Vaginal, IAP-	177/480 (36.9)	.06	28/473 (5.9)	.07	36/441 (8.2)	.10
Vaginal, IAP+	90/208 (43.3)		15/206 (7.3)		26/202 (12.9)	
Cesarean, scheduled	48/100 (48.0)		7/102 (6.9)		10/91 (11.0)	
Cesarean, emergency	59/127 (46.5)		16/125 (12.8)		17/113 (15.0)	
Infant sex, No. (%)						
Male	202/496 (40.7)	.86	46/493 (9.3)	.02	54/455 (11.9)	.13
Female	178/431 (41.3)		22/425 (5.2)		35/403 (8.7)	
Maternal race/ethnicity, No. (%)						
White	303/706 (42.9)	.01	53/699 (7.6)	.51	67/653 (10.3)	.15
Asian	40/98 (40.8)		8/96 (8.3)		14/89 (15.7)	
Other	36/125 (28.8)		6/124 (4.8)		9/120 (7.5)	
Maternal prenatal asthma, No. (%)						
No	342/854 (40.0)	.07	58/840 (6.9)	.07	77/790 (9.7)	.04
Yes	39/77 (50.6)		10/81 (12.3)		13/74 (17.6)	
Maternal prenatal allergy, No. (%)						
No	137/342 (40.1)	.64	23/338 (6.8)	.71	30/314 (9.6)	.47
Yes	236/567 (41.6)		42/563 (7.5)		59/530 (11.1)	
Maternal smoking during pregnancy, No. (%)						
No	353/875 (40.3)	.03	58/866 (6.7)	.005	85/817 (10.4)	.24
Yes	21/36 (58.3)		7/37 (18.9)		5/29 (17.2)	
Siblings, No. (%)						
No	175/445 (39.3)	.27	35/449 (7.8)	.70	49/426 (11.5)	.32
Yes	195/454 (43.0)		31/439 (7.1)		38/405 (9.4)	
Direct antibiotic exposure (0-12 mo)						
No	228/565 (40.3)	.09	39/557 (7.0)	.14	48/522 (9.2)	.006
Yes	78/163 (47.9)		18/167 (10.8)		27/155 (17.4)	
Breastfeeding status (0-3 mo), No. (%)						
No	86/154 (55.8)	<.001	17/153 (11.1)	.12	20/141 (14.2)	.06
Partial	127/279 (45.5)		22/282 (7.8)		32/258 (12.4)	
Exclusive	169/497 (34.0)		30/487 (6.2)		38/464 (8.2)	
Household pet exposure, No. (%)						
No	135/394 (34.3)	.001	24/393 (6.1)	.40	39/363 (10.7)	.28
Only prenatal	31/67 (46.3)		4/67 (6.0)		3/59 (5.1)	
Both prenatal and postnatal	189/405 (46.7)		34/401 (8.5)		46/383 (12.0)	
Maternal prepregnancy weight status, No. (%)						
Normal	NA	NA	22/522 (4.2)	<.001	24/507 (4.7)	<.001
OWOB	NA		45/371 (12.1)		63/328 (19.2)	

Abbreviations: IAP-, no intrapartum antibiotic prophylaxis; IAP+, intrapartum antibiotic prophylaxis; NA, not applicable; OWOB, overweight or obese.

of normal-weight mothers to have higher Firmicutes richness (eTable 5 in the Supplement). Jointly, maternal OWOB and emergency cesarean delivery were significantly associated with a higher abundance of fecal Lachnospiraceae in

infants (adjusted OR, 2.02; 95% CI, 1.06-3.87) and of several other microbiota at the family level. Few family-level differences in gut microbiota were found after maternal OWOB and vaginal birth.

Figure 1. Forest Plots Portraying Odds Ratios and 95% CIs of Factors Associated With Childhood Overweight or Obesity (OWOB)



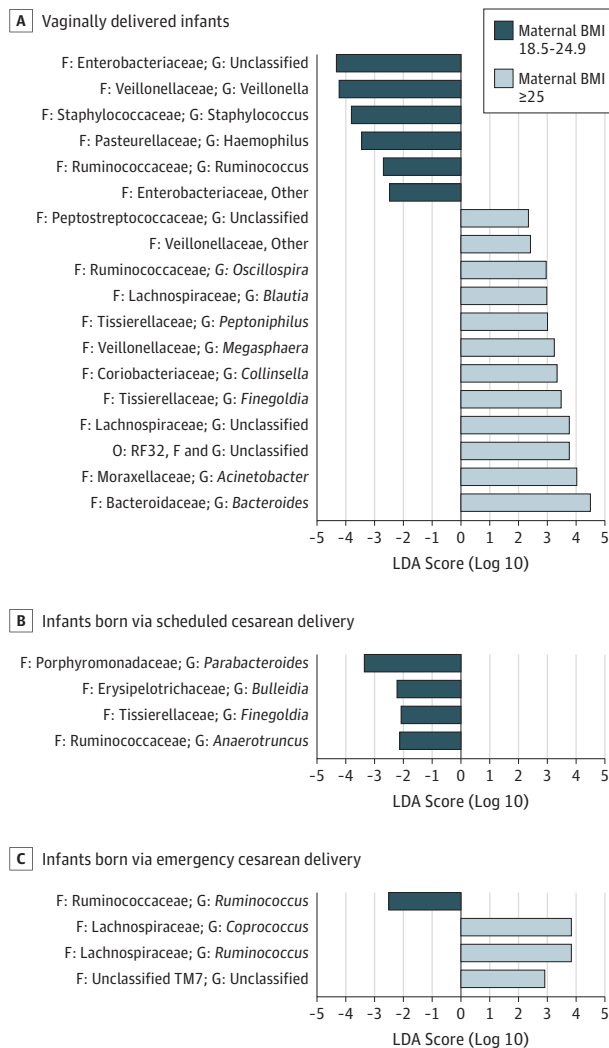
A, Odds ratios at age 1 year for childhood OWOB in association with maternal weight status, birth mode, and infant gut microbiota. B, Odds ratios at age 3 years for childhood OWOB in association with maternal weight status, birth mode, and infant gut microbiota. C, Joint associations at age 1 year of maternal weight status and birth mode. D, Joint associations at age 3 years of maternal weight status and birth mode. Adjusted (1): Adjusted for location, birth mode, infant sex, socioeconomic status (SES), maternal race/ethnicity, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12 months), and pet exposure. Adjusted (2): Adjusted for location, infant sex, SES, maternal weight status, maternal race/ethnicity, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12 months), and pet exposure. Adjusted (3): Adjusted for location, birth mode, infant sex, maternal weight status, maternal race/ethnicity, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12 months), pet exposure, and age at fecal sampling. Adjusted (4): Adjusted for location, infant sex, SES, maternal race/ethnicity, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12 months), and pet exposure. Error bars indicate 95% CIs. CD indicates cesarean delivery; IAP-, no intrapartum antibiotic prophylaxis; IAP+, intrapartum antibiotic prophylaxis; NW, normal-weight; and Ref, reference. The dotted lines indicate an odds ratio of 1.

Among vaginally born infants (Figure 2), the LefSe analysis revealed that infants born to OWOB vs normal-weight mothers had a statistically higher abundance of several genera (eg, *Bacteroides*, *Megasphaera*, *Blautia*, and *Oscillospira*) but reduced abundance of others (eg, *Haemophilus* and *Veillonella*). After emergency cesarean delivery, genera *Coprococcus* and *Ruminococcus* of the Lachnospiraceae family were more abundant in infants born to OWOB mothers than normal-weight mothers.

Sequential Mediators in the Intergenerational Transmission of OWOB

The sequential mediation model (Figure 3A) showed direct associations (path c') of prepregnancy OWOB with childhood OWOB and indirect associations (path a₁d₂₁b₂) with the sequential mediators, birth mode, and Firmicutes richness of the microbiota of the infant gut. Maternal OWOB was associated with cesarean delivery (path a₁) and cesarean delivery enriched Firmicutes richness (path d₂₁). A positive direct asso-

Figure 2. Differences in Relative Abundance of Bacterial Taxa in Microbiota of the Infant Gut Jointly Stratified by Delivery Mode and Maternal Prepregnancy Weight Status



Linear discriminant analysis (LDA) scores provided for differential taxon abundance between normal-weight mothers (BMI, 18.5-24.9 [dark blue]) and overweight mothers (BMI, ≥ 25 [light blue]). A, Vaginally delivered infants. B, Infants born via scheduled cesarean delivery. C, Infants born via emergency cesarean delivery. BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); F, family; G, genus; and O, order.

ciation of Firmicutes species richness with childhood OWOB was also observed (path b_2). Similar results were found for sequential mediation by birth mode and total species richness (eFigure 3 in the Supplement). The abundance of Lachnospiraceae in microbiota of the infant gut had a direct association with child OWOB at age 1 year (Figure 3B, path b_2 ; $\beta = 0.41$; $P < .05$). Sequential mediation involving birth mode and Lachnospiraceae abundance was identified in the pathway between maternal OWOB and child OWOB at age 1 year (path $a_1d_{21}b_2$; $\beta = 0.004$; 95% CI, 0.0001-0.0156). Direct and indirect associations between fecal Lachnospiraceae and OWOB at age 3 years were borderline. Other microbiota at the family level,

found to be associated with maternal OWOB or cesarean delivery, were not statistically significant mediators of the maternal and child OWOB association.

Microbiota Interaction Networks Associated With Child OWOB

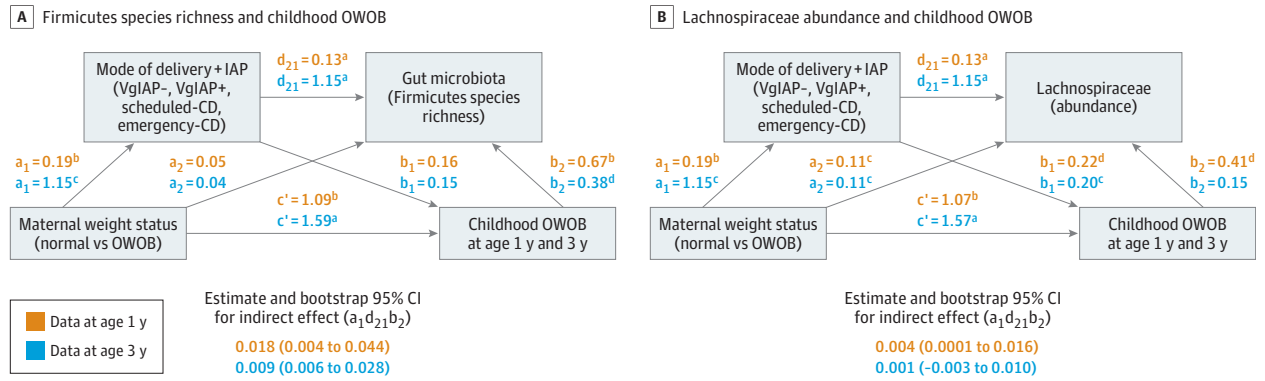
Compared with normal-weight infants, complex microbiota interaction networks involving more microbiota taxa were identified for OWOB children at ages 1 and 3 years regardless of their maternal weight status. However, among OWOB children born to OWOB mothers, the family Lachnospiraceae became more abundant with increasing levels of several other families or orders, including the Lactobacillales (lactic acid bacteria), Ruminococcaceae, and Veillonellaceae. Few of the same interactions for the family Lachnospiraceae were seen among OWOB children after a normal-weight pregnancy, and notably absent was a positive correlation with Lactobacillales (Figure 4 and eFigure 4 in the Supplement).

Discussion

In our general population birth cohort of 935 infants, those born to OWOB mothers were more likely to develop OWOB at ages 1 and 3 years, and the magnitude of the risk varied by birth mode. Compared with infants born vaginally after a normal-weight pregnancy, those born vaginally to OWOB mothers were 3.33 times more likely to become OWOB at age 1 year (95% CI, 1.49-7.41), whereas cesarean-delivered infants of OWOB mothers had a 5.02-fold risk of OWOB (95% CI, 2.04-12.38), when adjusted for other covariates. Infants born by emergency cesarean delivery to OWOB mothers were at highest risk for OWOB. Similar associations were found for OWOB at age 3 years. In keeping with the thesis of intergenerational transmission of obesogenic microbes,^{13,21,22} we found that enrichment of infant gut microbiota with the family Lachnospiraceae at ages 3 to 4 months mediated the association between maternal OWOB and child OWOB through a birth mode pathway. A mediation association for richness of total microbial species was also observed for child OWOB; this association also depended on birth mode, such that several microbial species (eg, *Bacteroides*) were more abundant in infants born vaginally to OWOB women. Our findings are consistent with those of other reports of overweight risk in preschool children following prepregnancy overweight^{35,36} and they extend our knowledge of causal pathways.

Among several emerging theories, the development of OWOB has been attributed to greater energy harvest from short-chain fatty acids produced by gut microbes when the abundance of Firmicutes exceeds that of the Bacteroidetes phylum.^{20,37} This thesis has found support in some,^{20,38} but not all,^{38,39} studies of obese children. We observed higher species richness and diversity within the Firmicutes phylum in infants born vaginally or by cesarean delivery to OWOB mothers, as well as evidence of mediation with this phylum. Within the Firmicutes phylum, experimental evidence is accumulating that the family Lachnospiraceae promotes adiposity,⁴⁰ inflammation in body fat,⁴¹ and the development of diabetes.^{42,43}

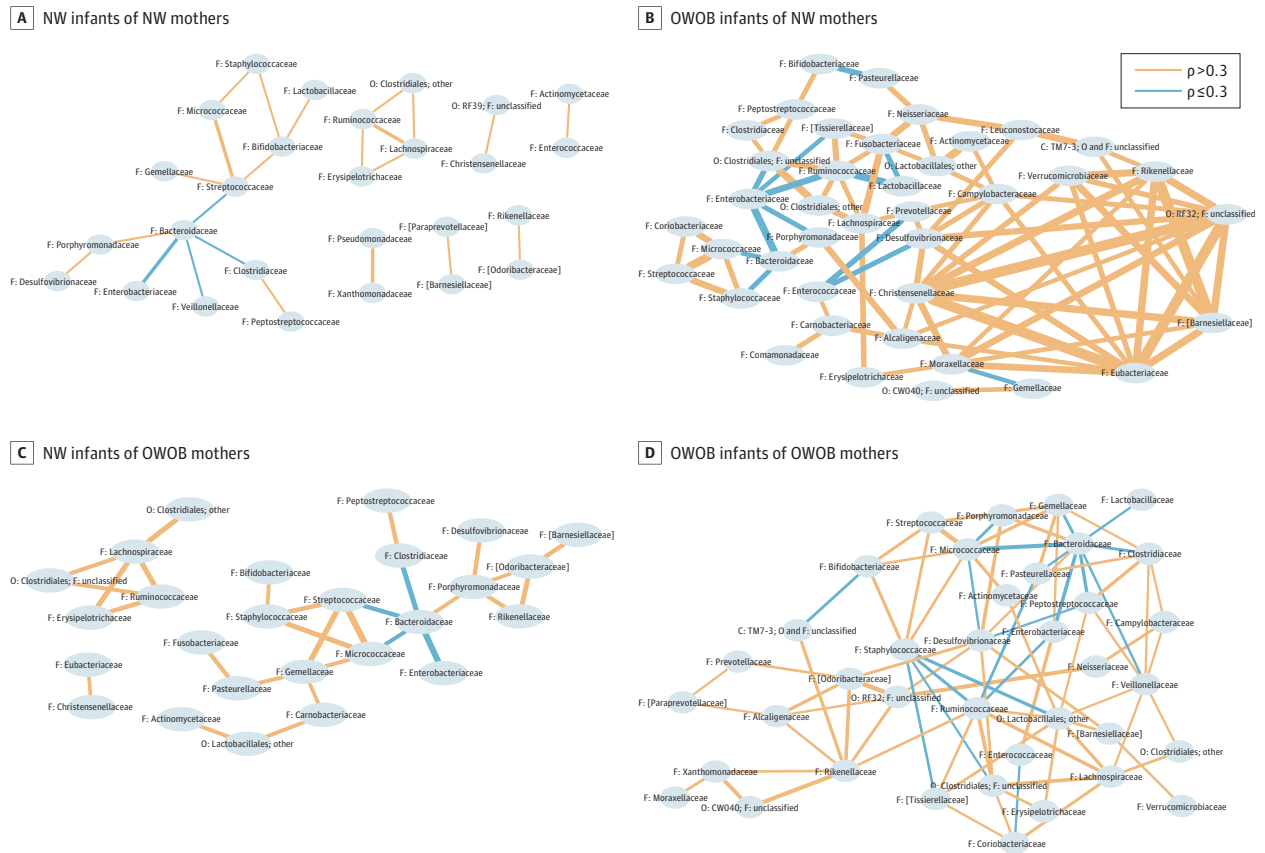
Figure 3. Sequential Mediation Models of Associations Between Maternal Weight Status, Modes of Delivery, and Microbiota of the Infant Gut



A, Firmicutes species richness and childhood overweight or obesity (OWOB). B, Lachnospiraceae abundance and childhood OWOB. CD indicates cesarean delivery; IAP, intrapartum antibiotic prophylaxis; VglAP-, vaginal delivery without IAP; and VglAP+, vaginal delivery with IAP.

^a $P < .001$.
^b $P < .01$.
^c $P < .10$.
^d $P < .05$.

Figure 4. Microbiota Interaction Networks for Groups of Overweight or Obese (OWOB) Phenotype Association Between Mothers and Offspring at Age 1 Year



A, Normal-weight (NW) infants of normal-weight mothers. B, OWOB infants of normal-weight mothers. C, Normal-weight infants of OWOB mothers. D, OWOB infants of OWOB mothers. C indicates class; F, family; and O, order. Connector

line thickness represents the value of the Spearman correlation coefficient (p), and brackets around the family name represent the proposed taxonomy by the Greengenes database.

We found indirect or mediating associations for infant fecal abundance of Lachnospiraceae in the association between maternal OWOB and child OWOB after vaginal or cesarean delivery. Within the Lachnospiraceae, genus *Blautia* was elevated among infants born vaginally to OWOB mothers, whereas abundance of *Coprococcus* and *Ruminococcus* was higher after prepregnancy OWOB in cesarean-delivered infants. We also found a direct association between infant fecal abundance of Lachnospiraceae and OWOB that was potentially initiated by postnatal factors affecting levels of these microbiota.

By including birth mode in a sequential mediation model, our study was able to determine whether mediating associations of gut microbiota were influenced by the birth process. With one exception,²² birth mode has not been accounted for in published studies of maternal overweight and microbiota of the child or infant gut.^{13,16,21,44} When newborns were stratified by birth mode, Mueller et al²² found higher fecal levels of *Bacteroides* only after vaginal birth in those born to mothers with prepregnancy OWOB. That study evaluated the composition of meconium soon after birth at a time when Lachnospiraceae are much less abundant. In agreement with the premise of the study by Mueller et al,²² but with results on the gut microbiota at 3 to 4 months after birth, our study points to birth mode as a major pathway for the association between maternal prepregnancy and child OWOB that involves Lachnospiraceae microbiota.

In addition to enrichment with Lachnospiraceae species, our biomarker analysis tool revealed greater prominence of genera belonging to *Acinetobacter*, *Bacteroides*, *Collinsella*, *Megasphaera*, *Finegoldia*, *Peptoniphilus*, and the Peptostreptococaceae family at ages 3 to 4 months in infants born vaginally to OWOB women. Most of these genera have been linked to obesity in adults⁴⁵⁻⁴⁸; the short-chain fatty acid metabolites they produce promote gluconeogenesis and fat storage.³⁷ In the network analysis we undertook in this study, multiple correlations between the abundance of individual taxa, including Lachnospiraceae, were apparent in OWOB children. Riva et al³⁸ also reported greater microbial taxon involvement with complex interaction networks in obese children. In our study, the abundance of Lachnospiraceae in OWOB children born to OWOB mothers increased with higher levels of several microbiota, notably of lactic acid bacteria, a correlation that was not seen in OWOB children born to normal-weight mothers. Hence, Lachnospiraceae microbes, which are known users of lactate,³⁷ may be essential to microbial interactions that initiate the OWOB phenotype in offspring, but the metabolic pathway may differ according to maternal weight status and the presence of other gut microbiota and their metabolites, such as lactate.

Because cesarean delivery is a key determinant of early gut dysbiosis in the infant, namely, delayed colonization of

Bacteroides,^{49,50} it is important to understand its association with risk of OWOB. Congruent with other cohorts, we found associations between maternal and child OWOB to be of greater magnitude for cesarean delivery than for vaginal delivery.⁷ As many have shown,^{11,51,52} maternal prepregnancy OWOB increased the likelihood of cesarean delivery in our study; this association was driven by emergency cesarean delivery. We observed many more perturbations to microbiota of the infant gut at the family level with maternal OWOB after cesarean delivery than after vaginal delivery; however, aside from Lachnospiraceae, these perturbations did not mediate the association between maternal and child OWOB. At the genus level, *Coprococcus* was associated with maternal prepregnancy OWOB in cesarean-delivered infants. A member of the Lachnospiraceae family, *Coprococcus* is more abundant in the gut of obese individuals,⁴⁷ including OWOB women during early pregnancy.⁵³

Strengths and Limitations

Our study has several strengths, including the application of high-throughput deep sequencing to profile gut microbiota in a birth cohort, with a representative and large sample size. We pioneered tests of sequential mediation involving birth mode and infant gut microbial diversity. On the other hand, 16S ribosomal RNA sequencing in our study may have underrepresented organisms such as the bifidobacteria. Because the association of breastfeeding and antibiotic exposure with microbiota of the infant gut and childhood OWOB was reported previously,^{54,55} we adjusted for those covariates; further study is needed to examine the roles of these covariates in the sequential mediator pathway proposed in this study. The role of maternal microbiota (gut, vaginal, and skin) and of gut microbiota in older children in the intergenerational transmission of OWOB deserves further attention.

Conclusions

Both maternal weight status and cesarean delivery shape early-life gut microbial development and the weight outcome of offspring, for which a mediation role for gut microbiota has been posited. In this large prospective cohort, we found evidence of a novel sequential mediator pathway involving birth mode and Firmicutes species richness of microbiota of the infant gut, namely, higher abundance of Lachnospiraceae for the intergenerational transmission of OWOB. The mediation association was evident in vaginal and cesarean delivery, but vaginal birth yielded a lower risk for child OWOB than did cesarean delivery.

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