

Supplementary Online Content

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This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix. Detailed Methods

Fecal microbiota analysis

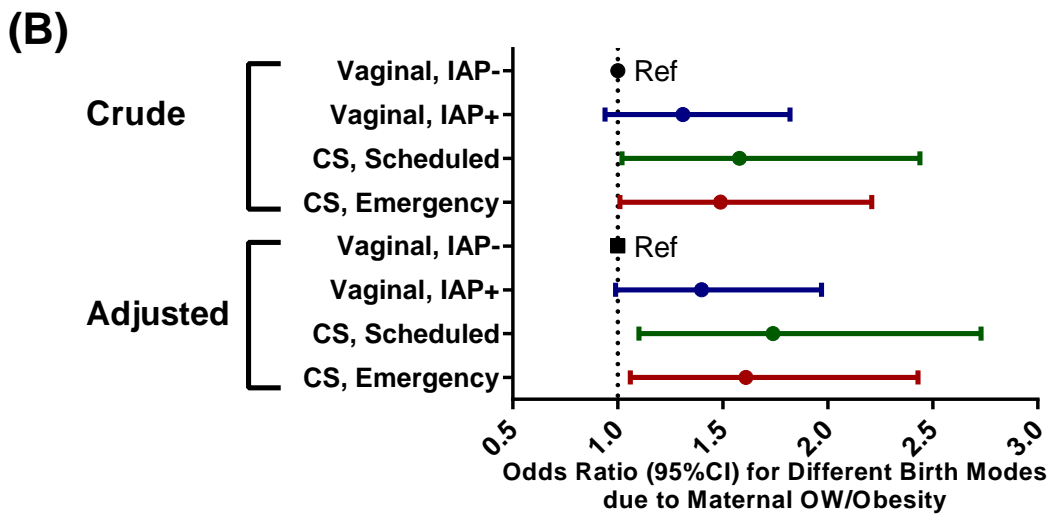
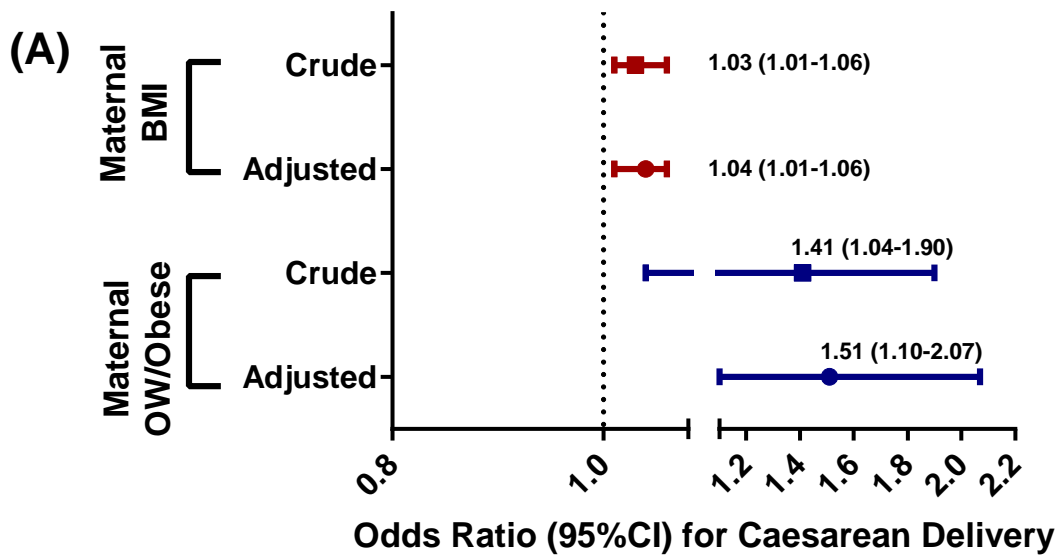
Samples were refrigerated immediately after collection and during transport and stored at -80°C until analysis. Genomic DNA was extracted from 80 to 200 mg of stool using the QIAamp DNA Stool Mini kit (Qiagen, Venlo, the Netherlands). The V4 hypervariable region of the bacterial 16S rRNA gene was amplified by PCR using universal bacterial primers: V4-515f: 5' AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT ATG GTA ATT GTG TGC CAG CMG CCG CGG TAA-3' , V4-806r:5' -CAA GCA GAA GAC GGC ATA CGA GAT XXXXXXXXXXXXX AGT CAG TCA GCC GGA CTA CHV GGG TWT CTA AT-3. For sample multiplexing, reverse primers were barcoded uniquely for each sample (barcoded sequence was denoted in the primer sequence by Xs). Each 25 μl PCR mixture contained 12.5 μl 2x Kapa2G Hotstart mix (Kapa Biosystems, Wilmington, MA), 0.6 μM of both forward and reverse primers and 2 μl genomic DNA (5 ng/ μl). PCR amplification consisted of an initial denaturation step for 3 min at 94°C , followed by 20 cycles of denaturation for 30 s at 94°C , annealing for 30 s at 50°C and an extension step for 30 s at 72°C . PCR reactions for each sample were performed in triplicate with a negative control in each run. One hundred nanograms of pooled PCR product from each sample was concentrated using an Amicon Ultra-4 30K centrifugal filter (Millipore, Billerica, MA, USA), run on a 1.4% agarose gel, extracted and cleaned with the GENE-CLEAN Turbo Kit (MP Biomedicals Inc, Solon, OH, USA). Pooled PCR amplicons were subjected to paired-end sequencing by Illumina Miseq platform.

Bioinformatic analysis

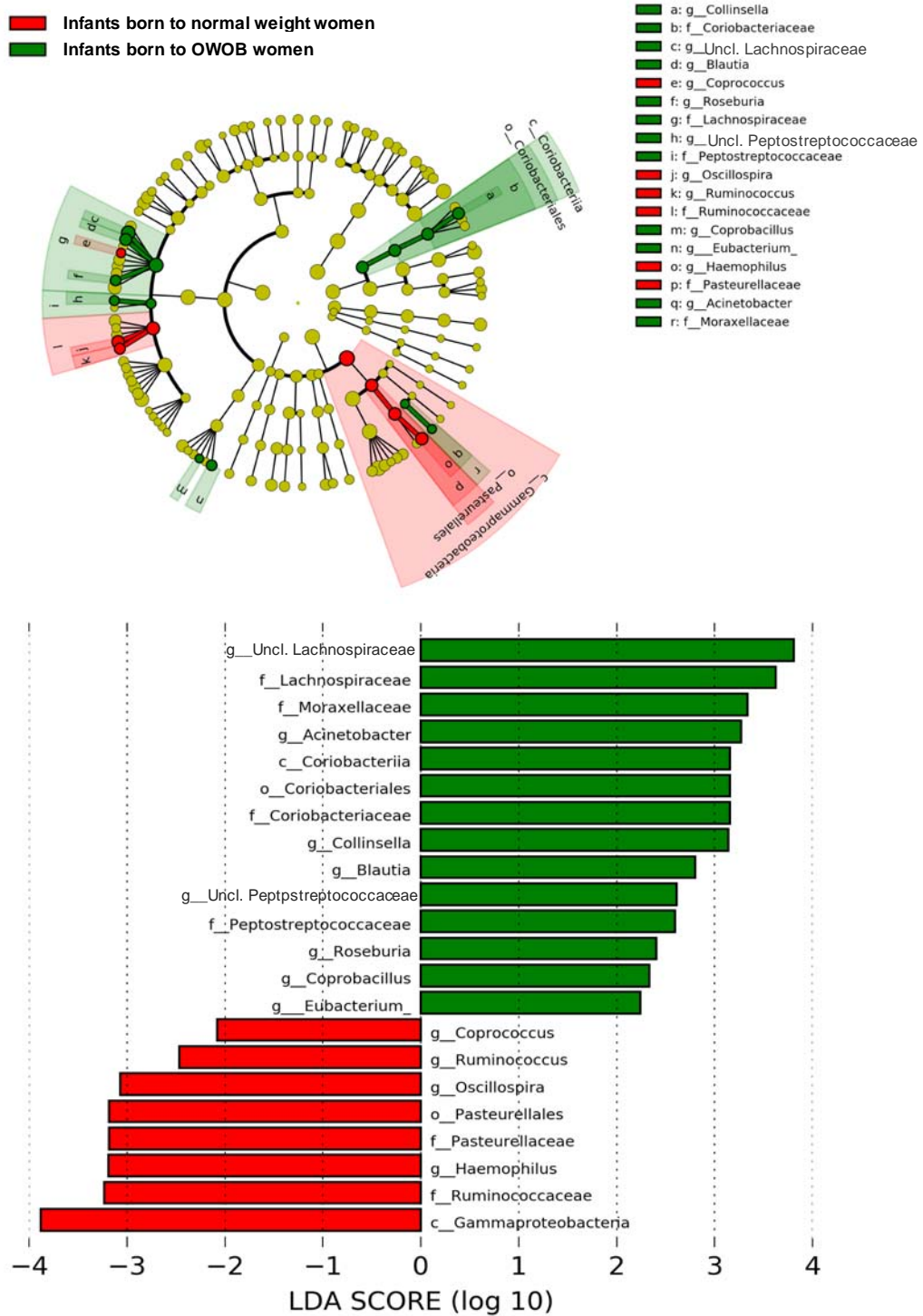
Using a QIIME pipeline (v1.9.0, qiime.org)¹, forward and reverse reads were assembled using PandaSeq for a final length of 144 bp (unassemblable sequences discarded), demultiplexed and filtered against the GREENGENES reference database (v13.8)² to remove all sequences with <60% similarity. Remaining sequences were clustered with Usearch61 at 97% sequence similarity against the GREENGENES database (closed picking algorithm), and taxonomic assignment was achieved using the RDP classifier³ constrained by GREENGENES. After taxonomic assignment, operational taxonomic units (OTUs) representing bacterial origin were selected, and bacterial OTUs with overall relative abundance below 0.0001 were excluded from subsequent downstream analyses. To avoid the bias due to variation in sequencing depths among samples, data were rarefied to 13,000 sequences per sample.

With the recommended pipeline in QIIME, relative abundance of bacterial OTUs was summarized at the phylum, family and genus levels. Microbial alpha diversity within samples was calculated with three standard indices (Chao1, Shannon and Simpson). Microbial community differences between samples (beta diversity) were examined by the permutational multivariate analysis of variance (PERMANOVA) comparison of both unweighted and weighted UNIFRAC distance matrices, with 1000 permutations.

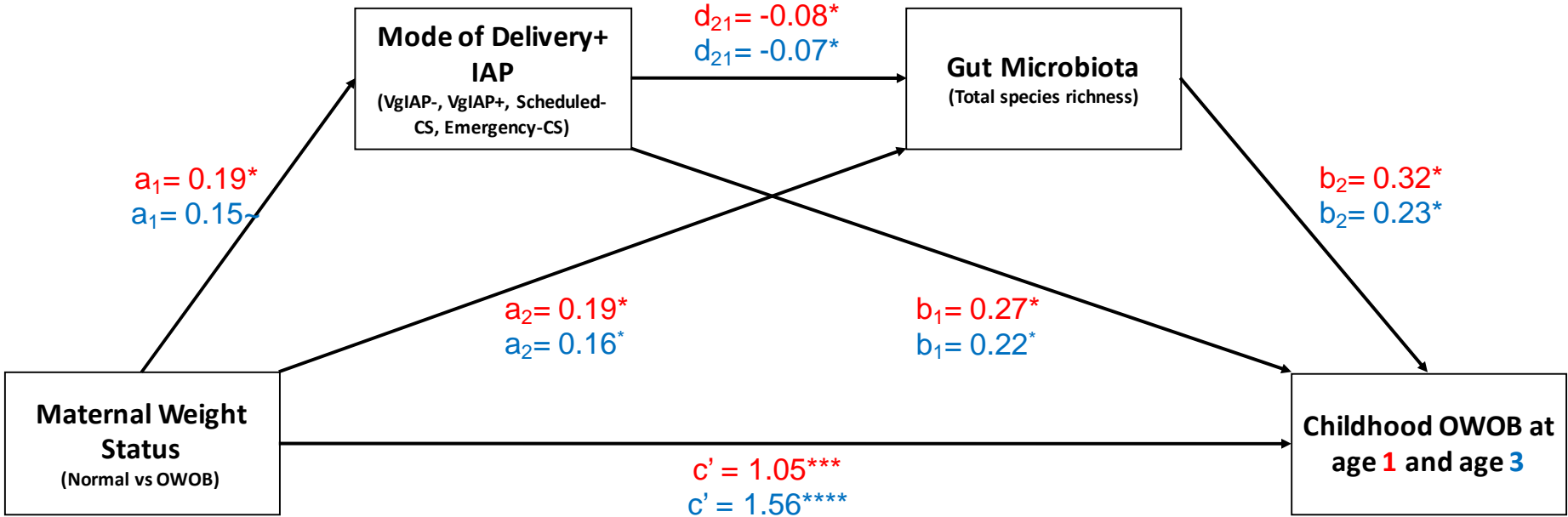
eFigure 1. Odds Ratios for (A) Caesarean Delivery and (B) Various Birth Modes, in Relation With Infants Born to OWOB Mothers



eFigure 2. Linear Discriminant Analysis (LDA) Scores for Differentially Abundant of Bacterial Taxa Due to Maternal Weight Status ($P < .05$)



eFigure 3. A Sequential Mediation Model of Associations Between Maternal Weight Status, Modes of Delivery, Infant Gut Microbiota (Total Species Richness), and Childhood OWOB

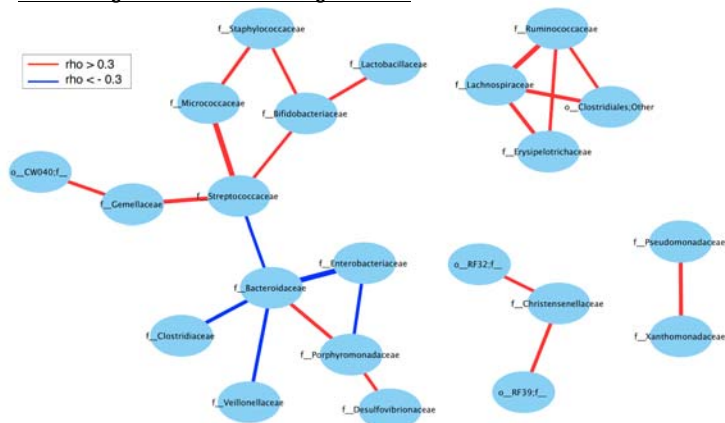


Bootstrap 95%CI for indirect effect ($a_1 d_{21} b_{20}$)
 [-0.017, -0.0006]
 [-0.012, -0.0001]

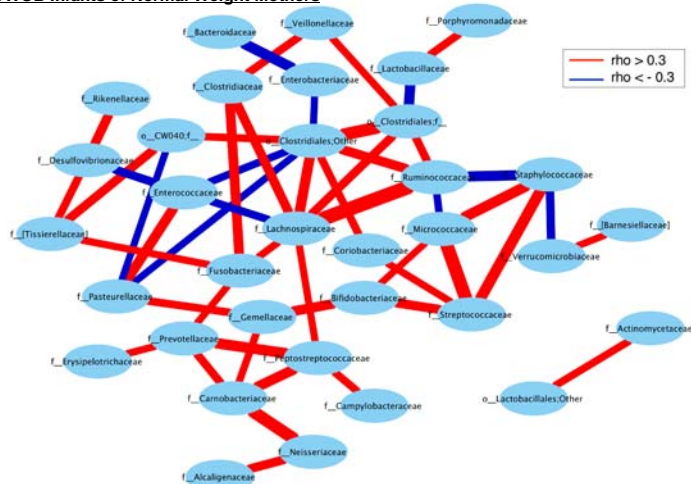
- $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$

eFigure 4. Microbiota Interaction Networks for Groups of OWOB Phenotype Relationship Between Mothers and Offspring at Age 3 Years

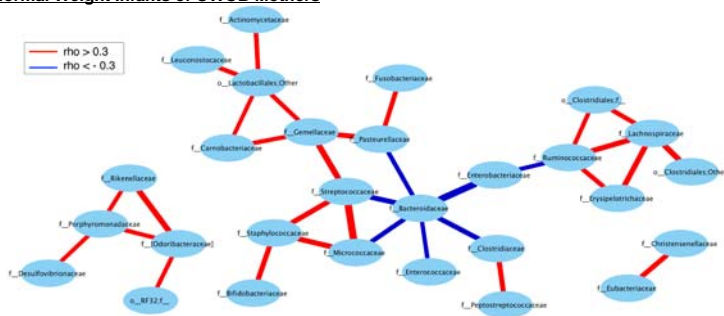
Normal Weight Infants of Normal Weight Mothers



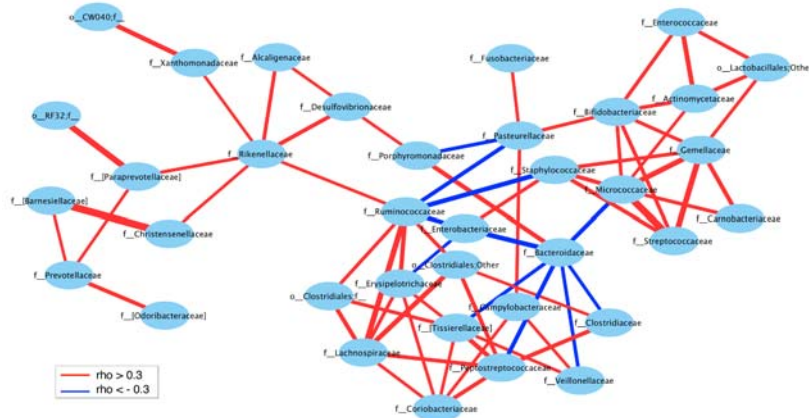
OWOB Infants of Normal Weight Mothers



Normal Weight Infants of OWOB Mothers



OWOB Infants of OWOB Mothers



eTable 1. Odds Ratios for Childhood OWOB at Age 1 and 3 in Relation to Maternal Weight Status, Birth Modes, and Infant Gut Microbiota

		Child OWOB at 1 year		Child OWOB at 3 years	
		OR (95%CI)	P value	OR (95%CI)	P value
Maternal OWOB					
Crude Model		3.14 (1.85-5.32)	<0.0001	4.78 (2.92-7.84)	<0.0001
Adjusted Model ¹		3.80 (1.88-7.66)	<0.0001	3.79 (2.10-6.84)	<0.0001
Maternal BMI Z-score					
Crude Model		1.06 (1.02-1.10)	0.001	1.12 (1.08-1.16)	<0.0001
Adjusted Model ¹		1.06 (1.01-1.12)	0.015	1.09 (1.05-1.14)	<0.0001
Chao1 (below vs above medium)					
Crude Model		2.12 (1.26-3.56)	0.004	1.63 (1.04-2.55)	0.032
Adjusted Model ²		1.65 (0.91-3.01)	0.101	1.32 (0.79-2.21)	0.294
Chao1 Firmicutes (below vs above medium)					
Crude Model		2.36 (1.39-4.00)	0.002	1.84 (1.17-2.90)	0.008
Adjusted Model ²		1.90 (1.01-3.59)	0.047	1.49 (0.87-2.55)	1.151
Shannon Firmicutes (below vs above medium)					
Crude Model		1.96 (1.17-3.27)	0.01	1.97 (1.25-3.10)	0.003
Adjusted Model ²		1.41 (0.76-2.62)	0.198	1.51 (0.89-2.57)	0.128
Birth Modes					
Crude Model	Vaginal, IAP-	Ref		Ref	
	Vaginal, IAP+	1.25 (0.65-2.39)	0.504	1.66 (0.97-2.84)	0.063
	Caesarean, scheduled	1.17 (0.50-2.76)	0.718	1.39 (0.66-2.91)	0.384
	Caesarean, emergency	2.33 (1.22-4.46)	0.011	1.99 (1.07-3.70)	0.029
Adjusted Model ³	Vaginal, IAP-	Ref		Ref	
	Vaginal, IAP+	1.01 (0.46-2.26)	0.973	1.59 (0.82-3.05)	0.168
	Caesarean, scheduled	0.80 (0.28-2.27)	0.669	1.33 (0.56-3.14)	0.516
	Caesarean, emergency	1.89 (0.83-4.33)	0.132	1.95 (0.88-4.30)	0.098
Maternal OWOB and Caesarean Delivery					
Crude Model	NW Mother and Vaginal Birth	Ref		Ref	
	NW Mother and CS	1.30 (0.50-3.41)	0.590	0.72 (0.24-2.16)	0.559
	OWOB Mother and Vaginal Birth	2.77 (1.46-5.28)	0.002	4.10 (2.31-7.27)	<0.0001
	OWOB Mother and CS	4.78 (2.33-9.83)	<0.0001	7.30 (3.76-14.18)	<0.0001
Adjusted Model ⁴	NW Mother and Vaginal Birth	Ref		Ref	
	NW Mother and CS	0.87 (0.23-3.27)	0.831	0.58 (0.16-2.07)	0.400
	OWOB Mother and Vaginal Birth	3.33 (1.49-7.41)	0.003	3.07 (1.58-5.96)	0.001
	OWOB Mother and CS	5.02 (2.04-12.38)	<0.0001	5.55 (2.55-12.04)	<0.0001
Maternal OWOB and Emergency CS Delivery[#]					
Crude Model	NW Mother and Vaginal Birth	Ref		Ref	
	NW Mother and Emergency CS	2.28 (0.81-6.45)	0.121	1.09 (0.31-3.79)	0.890
	OWOB Mother and Vaginal Birth	3.14 (1.69-5.83)	<0.0001	4.54 (2.63-7.83)	<0.0001
	OWOB Mother and Emergency CS	6.43 (2.81-14.68)	<0.0001	8.53 (3.97-18.40)	<0.0001
Adjusted Model ⁴	NW Mother and Vaginal Birth	Ref		Ref	
	NW Mother and Emergency CS	1.12 (0.23-5.36)	0.892	0.85 (0.18-3.95)	0.837
	OWOB Mother and Vaginal Birth	3.27 (1.54-6.95)	0.002	3.44 (1.84-6.44)	<0.0001
	OWOB Mother and Emergency CS	7.92 (2.85-21.98)	<0.0001	7.10 (2.76-18.26)	<0.0001

¹Adjusted for location, birth modes, infant sex, socioeconomic status (SES), maternal race, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12months), and pet exposure.

²Adjusted for location, birth modes, infant sex, maternal weight status, maternal race, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12months), pet exposure, and age at fecal sampling.

³Adjusted for location, infant sex, SES, maternal weight status, maternal race, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12months), and pet exposure.

⁴Adjusted for location, infant sex, SES, maternal race, maternal prenatal asthma, maternal prenatal smoking, breastfeeding status, oral antibiotic use (0-12months), and pet exposure.

[#]Only emergency CS was included in the model because it drove the CS association with child OWOB.

eTable 2. Significant Microbiota Measurements in Infant Gut at 3-4 Months, According to Maternal Weight Status ($P<.05$)

	NW Mother N=553 Median (IQR)	OFOB Mother N=382 Median (IQR)	P Value
Diversity			
Chao1	193.30 (168.42-226.88)	202.77 (174.11-232.37)	.005
Chao1 Firmicutes	73.00 (50.69-94.38)	75.05 (57.45-101.93)	.015
Shannon Firmicutes	2.54 (1.97-3.06)	2.77 (2.23-3.25)	<.001
Phyla			
Proteobacteria	19.66 (9.70-40.41)	16.68 (5.73-38.97)	.015
Families			
Coriobacteriaceae	0.04 (0.01-0.14)	0.05 (0.01-0.30)	<.001
Erysipelotrichaceae	0.02 (0.00-0.31)	0.04 (0.00-0.35)	.032
Lachnospiraceae	2.04 (0.03-9.83)	3.56 (0.24-9.70)	.043
Ruminococcaceae	0.05 (0.00-1.51)	0.28 (0.01-2.03)	.002
Enterobacteriaceae	18.05 (7.52-38.82)	14.70 (4.16-37.39)	.009
Pasteurellaceae	0.05 (0.00-0.41)	0.02 (0.00-0.22)	.001
Ratios			
E/B ratio	1.78 (0.19-380.32)	0.80 (0.10-289.73)	.036

eTable 3. Significant Microbiota Measurements in Infant Gut at 3-4 Months, According to Joint Effects of Maternal Prepregnancy OWOB and Emergency CS ($P<.05$)

Microbiota Measurements	NW Mother and Vaginal Birth N=421 Median (IQR)	NW Mother and Emergency CS N=68 Median (IQR)	OWOB Mother and Vaginal Birth N=267 Median (IQR)	OWOB Mother and Emergency CS N=59 Median (IQR)	P Value
Diversities					
Chao1	194.79 (170.11-229.09) ^a	186.23 (149.66-224.82) ^a	204.00 (177.30-237.04) ^b	207.14 (177.05-229.52) ^{ab}	.009
Chao1 Firmicutes	71.33 (48.88-91.39) ^a	81.25 (55.93-110.91) ^b	71.60 (54.38-93.00) ^a	90.11 (68.67-117.50) ^b	<.001
Shannon Firmicutes	2.51 (1.91-3.07) ^a	2.58 (1.96-3.08) ^a	2.73 (2.21-3.29) ^b	2.72 (2.20-3.08) ^b	.004
Chao1 Bacteroidetes	48.75 (13.13-77.63) ^a	12.00 (5.13-33.00) ^b	56.67 (21.00-90.08) ^c	11.00 (6.00-40.50) ^b	<.001
Chao1 Proteobacteria	55.75 (40.29-74.00) ^a	63.79 (47.57-79.52) ^b	56.00 (35.00-71.09) ^a	68.00 (54.75-85.09) ^b	<.001
Phyla					
Bacteroidetes	27.99 (0.17-62.63) ^a	0.12 (0.04-1.15) ^b	44.85 (0.53-70.30) ^c	0.11 (0.06-4.15) ^b	<.001
Firmicutes	20.71 (7.79-44.11) ^a	33.99 (21.72-66.84) ^b	16.14 (7.06-33.16) ^a	40.70 (17.80-57.94) ^b	<.001
Proteobacteria	17.44 (9.27-39.70) ^a	25.32 (12.51-50.49) ^b	14.26 (4.78-33.30) ^c	31.82 (13.84-48.77) ^b	<.001
Families					
<i>Actinomycetaceae</i>	0.02 (0.00-0.09) ^a	0.03 (0.00-0.09) ^b	0.02 (0.00-0.10) ^a	0.04 (0.02-0.13) ^b	.029
<i>Coriobacteriaceae</i>	0.03 (0.00-0.12) ^a	0.04 (0.01-0.12) ^a	0.05 (0.01-0.33) ^b	0.05 (0.01-0.44) ^b	.009
<i>Bacteroidaceae</i>	23.99 (0.12-59.50) ^a	0.10 (0.03-0.64) ^b	37.10 (0.36-65.73) ^c	0.09 (0.03-1.63) ^b	<.001
<i>Clostridiaceae</i>	0.23 (0.02-2.41) ^a	1.48 (0.12-5.88) ^b	0.24 (0.03-1.37) ^a	1.20 (0.30-6.15) ^b	<.001
<i>Enterococcaceae</i>	0.02 (0.00-0.09) ^a	0.05 (0.02-0.12) ^b	0.02 (0.00-0.05) ^a	0.07 (0.02-0.22) ^b	<.001
<i>Lachnospiraceae</i>	1.79 (0.03-8.82) ^a	4.21 (0.02-14.38) ^b	2.59 (0.25-8.55) ^{ab}	5.59 (0.21-14.44) ^b	.022
<i>Ruminococcaceae</i>	0.04 (0.00-1.37) ^a	0.07 (0.01-3.02) ^a	0.32 (0.01-2.08) ^{ab}	0.07 (0.01-1.62) ^a	.007
<i>Streptococcaceae</i>	0.57 (0.19-1.75) ^a	0.86 (0.32-2.13) ^b	0.46 (0.16-1.62) ^a	1.05 (0.41-2.28) ^b	.007
<i>Veillonellaceae</i>	3.64 (0.55-16.85) ^a	10.59 (3.86-27.00) ^b	3.61 (0.66-12.13) ^a	11.67 (1.51-30.40) ^b	<.001
<i>Enterobacteriaceae</i>	15.69 (6.95-36.63) ^a	24.57 (10.79-49.97) ^b	10.51 (3.45-30.28) ^c	29.23 (11.27-48.35) ^b	<.001
<i>Pasteurellaceae</i>	0.05 (0.00-0.39) ^a	0.08 (0.00-0.87) ^a	0.02 (0.00-0.12) ^b	0.05 (0.00-0.55) ^a	<.001
Ratios					
F/B ratio	0.81 (0.17-105.00) ^a	201.49 (15.56-1062.60) ^b	0.48 (0.13-23.27) ^c	295.32 (8.31-810.13) ^b	<.001
E/B ratio	0.78 (0.15-163.67) ^a	213.90 (7.61-945.51) ^b	0.37 (0.07-51.01) ^c	221.17 (6.06-851.22) ^b	<.001

^{a-c}Means within a row with different superscripts differ $P<.05$ in Dunn's post hoc tests.

eTable 4. Crude and Adjusted Likelihoods of Infant Gut Microbiota Measurements at 3-4 Months in Relation to Maternal OWOB

Microbiota Measurements	Maternal OWOB	
	Crude Model OR (95% CI)	Adjusted Model [#] aOR (95%CI)
Chao1	1.45 (1.12-1.89)	1.26 (0.93-1.69)
Shannon Firmicutes	1.53 (1.18-1.99)	1.19 (0.88-1.61)
Coriobacteriaceae	1.30 (1.00-1.69)	1.27 (0.89-1.83)
Erysipelotrichaceae	1.30 (1.00-1.69)	0.94 (0.69-1.28)
Lachnospiraceae	1.31 (1.01-1.70)	0.84 (0.61-1.15)
Ruminococcaceae	1.60 (1.23-2.08)	1.09 (0.79-1.50)
Pasteurellaceae	0.69 (0.53-0.90)	0.83 (0.61-1.13)
E/B ratio	0.77 (0.59-1.00)	0.71 (0.52-0.97)

[#]Adjusted for birth modes, infant sex, maternal race, breastfeeding status, direct antibiotic use (0-3 months), maternal prenatal asthma, maternal prenatal smoking, pet exposure and age at fecal sampling.

eTable 5. Crude and Adjusted Likelihoods of Infant Gut Microbiota Measurements at 3-4 Months in Relation to Maternal Prepregnancy OWOB and Emergency Cesarean Delivery

Microbiota Measurement	NW Mother and Vaginal	NW Mother and Emergency CS	OWOB Mother and Vaginal	OWOB Mother and Emergency CS
Chao1				
Crude Model	Ref	0.60 (0.35-1.02)	1.44 (1.06-1.96)	2.02 (1.14-1.75)
Adjusted Model [#]	Ref	0.55 (0.30-1.01)	1.42 (0.97-2.08)	1.73 (0.93-3.22)
Chao1 Firmicutes				
Crude Model	Ref	1.44 (0.86-2.41)	1.01 (0.75-1.38)	2.59 (1.44-4.66)
Adjusted Model [#]	Ref	1.61 (0.89-2.91)	0.75 (0.51-1.12)	2.17 (1.14-4.14)
Shannon Firmicutes				
Crude Model	Ref	1.16 (0.69-1.94)	1.57 (1.15-2.13)	1.37 (0.80-2.37)
Adjusted Model [#]	Ref	1.37 (0.76-2.47)	1.22 (0.82-1.80)	1.23 (0.66-2.28)
Chao1 Bacteroidetes				
Crude Model	Ref	0.15 (0.08-0.29)	1.50 (1.10-2.06)	0.26 (0.14-0.49)
Adjusted Model [#]	Ref	0.10 (0.05-0.23)	1.45 (0.98-2.15)	0.20 (0.10-0.40)
Chao1 Proteobacteria				
Crude Model	Ref	2.17 (1.27-3.69)	1.08 (0.80-1.47)	2.30 (1.33-4.08)
Adjusted Model [#]	Ref	2.00 (1.13-3.55)	1.65 (1.13-2.41)	2.68 (1.45-4.92)
Bacteroidetes				
Crude Model	Ref	0.21 (0.11-0.39)	1.60 (1.17-2.20)	0.17 (0.08-0.33)
Adjusted Model [#]	Ref	0.18 (0.09-0.36)	1.68 (1.13-2.49)	0.15 (0.07-0.31)
Firmicutes				
Crude Model	Ref	2.91 (1.65-5.10)	0.77 (0.56-1.04)	3.03 (1.65-5.54)
Adjusted Model [#]	Ref	2.54 (1.40-4.59)	0.77 (0.53-1.12)	3.07 (1.61-5.84)
Proteobacteria				
Crude Model	Ref	1.87 (1.10-3.16)	0.72 (0.53-0.98)	2.11 (1.19-3.75)
Adjusted Model [#]	Ref	1.74 (0.97-3.12)	0.90 (0.61-1.32)	2.72 (1.46-5.09)
Bacteroidaceae				
Crude Model	Ref	0.16 (0.08-0.31)	1.50 (1.09-2.06)	0.15 (0.08-0.31)
Adjusted Model [#]	Ref	0.14 (0.07-0.29)	1.58 (1.07-2.33)	0.14 (0.07-0.30)
Enterococcaceae				
Crude Model	Ref	2.55 (1.47-4.45)	0.86 (0.63-1.17)	2.07 (1.17-3.68)
Adjusted Model [#]	Ref	2.54 (1.41-4.56)	0.98 (0.67-1.41)	2.05 (1.13-3.73)
Clostridiaceae				
Crude Model	Ref	2.97 (1.71-5.19)	1.00 (0.73-1.35)	2.82 (1.57-5.08)
Adjusted Model [#]	Ref	2.59 (1.45-4.63)	0.88 (0.60-1.27)	2.66 (1.43-4.93)
Lachnospiraceae				
Crude Model	Ref	1.45 (0.87-2.43)	1.24 (0.91-1.68)	2.56 (1.44-4.57)
Adjusted Model [#]	Ref	1.50 (0.82-2.74)	0.84 (0.57-1.25)	2.02 (1.06-3.87)
Ruminococcaceae				
Crude Model	Ref	1.26 (0.78-2.11)	1.92 (1.41-2.61)	1.14 (0.66-1.97)
Adjusted Model [#]	Ref	1.43 (0.77-2.67)	1.36 (0.91-2.03)	0.77 (0.40-1.48)
Veillonellaceae				
Crude Model	Ref	2.85 (1.62-5.01)	0.87 (0.64-1.19)	2.52 (1.40-4.52)
Adjusted Model [#]	Ref	3.03 (1.64-5.59)	0.75 (0.52-1.10)	2.47 (1.32-4.61)
Enterobacteriaceae				
Crude Model	Ref	2.10 (1.23-3.60)	0.71 (0.53-0.97)	1.81 (1.03-3.17)
Adjusted Model [#]	Ref	1.98 (1.09-3.59)	0.89 (0.61-1.30)	2.25 (1.22-4.16)
Pasteurellaceae				
Crude Model	Ref	1.17 (0.70-1.97)	0.59 (0.43-0.80)	0.90 (0.52-1.55)
Adjusted Model [#]	Ref	1.17 (0.65-2.09)	0.67 (0.45-1.00)	1.36 (0.73-2.53)
F/B ratio				
Crude Model	Ref	5.69 (2.96-10.94)	0.65 (0.47-0.89)	6.74 (3.22-14.09)
Adjusted Model [#]	Ref	6.72 (3.23-14.00)	0.59 (0.40-0.88)	7.30 (3.39-15.74)
E/B ratio				
Crude Model	Ref	4.45 (2.39-8.23)	0.59 (0.43-0.81)	4.91 (2.47-9.75)
Adjusted Model [#]	Ref	4.95 (2.48-9.84)	0.63 (0.43-0.93)	5.30 (2.57-10.93)

[#]Adjusted for infant sex, maternal race, breastfeeding status, direct antibiotic use (0-3 months), maternal prenatal asthma, maternal prenatal smoking, pet exposure and age at fecal sampling.

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