Prenatal exposure to antibiotics, cesarean section, and risk of childhood obesity


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Title: Prenatal exposure to antibiotics, cesarean section, and risk of childhood obesity

Authors: *Noel T. Mueller1,2, Robin Whyatt3, Lori Hoepner3, Sharon Oberfield4, Maria Gloria Dominguez-Bello5, Elizabeth M. Widen1,2, Abeer Hassoun4, Frederica Perera3, Andrew Rundle1

1Department of Epidemiology, Mailman School of Public Health, Columbia University Medical Center, New York, NY, USA
2Institute of Human Nutrition and Department of Medicine, College of Physicians and Surgeons, Columbia University Medical Center, New York, NY, USA
3Department of Environmental Health Sciences, Mailman School of Public Health, Columbia University Medical Center, New York, NY, USA
4Department of Pediatrics, New York-Presbyterian Hospital, Columbia University Medical Center, New York, NY, USA
5Division of Translational Medicine, New York University School of Medicine, New York, NY, USA

*Corresponding Author:
Noel Theodore Mueller
Department of Epidemiology, Mailman School of Public Health, Columbia University Medical Center, New York, NY 10002
Phone: 414-779-1167
E-mail: nm2768@columbia.edu

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ABSTRACT

BACKGROUND/OBJECTIVES: Cesarean section (CS) and antibiotic use during pregnancy may alter normal maternal-fetal microbiota exchange, thereby contributing to aberrant microbial colonization of the infant gut and increased susceptibility to obesity later in life. We hypothesized that i) maternal use of antibiotics in the second or third trimester of pregnancy and ii) CS are independently associated with higher risk of childhood obesity in the offspring.

SUBJECTS/METHODS: Of the 727 mothers enrolled in the Northern Manhattan Mothers and Children Study, we analyzed the 436 mother-child dyads followed until 7 years of age with complete data. We ascertained prenatal antibiotic by a questionnaire administered late in the third trimester, and delivery mode by medical record. We derived age- and sex-specific BMI $z$ scores using the CDC SAS Macro, and defined obesity as BMI $z \geq 95^{\text{th}}$ percentile. We used binary regression with robust variance and linear regression models adjusted for maternal age, ethnicity, pre-gravid BMI, maternal receipt of public assistance, birth weight, sex, breast feeding in the first year, and gestational antibiotics or delivery mode.

RESULTS: Compared to children not exposed to antibiotics during the second or third trimester, those exposed had 84% (33-154%) higher risk of obesity, after multivariable adjustment. Second or third trimester antibiotic exposure was also positively associated with BMI $z$ scores, waist circumference, and % body fat (all $p < 0.05$). Independent of prenatal antibiotic usage, CS was associated with 46% (8-98%) higher offspring risk of childhood obesity. Associations were similar for elective and non-elective CS.

CONCLUSIONS: In our cohort, CS and exposure to antibiotics in the second or third trimester were associated with higher offspring risk of childhood obesity. Future studies that address the
limitations of our study are warranted to determine if prenatal antibiotic use is associated with offspring obesity. Research is also needed to determine if alterations in neonatal gut microbiota underlie the observed associations.
BACKGROUND

The commensal bacteria (microbiota) in the human intestine play an important role in adipogenesis. Cesarean section (CS) and early-life antibiotics deprive normal microbial colonization of the newborn gut. Mounting evidence also links these exposures to development of obesity later in life.

However, important questions remain. First, while meta-analyses have concluded that CS compared to vaginal delivery (VD) is associated with greater risk of obesity in children, adolescents, and adults, none of the studies included in these meta-analyses simultaneously controlled for differences in pre-gravid maternal body mass index (BMI), birth weight, breastfeeding, and gestational antibiotics—all potential confounders to the CS-obesity association. Second, studies on early-life antibiotics and childhood obesity have thus far focused on postnatal exposure to antibiotics, with one of these studies reporting that earlier (<6 months) but not later (≥6 months) administration of antibiotics was associated with higher body mass from 10 to 38 months. No studies have reported on whether maternal antibiotic use in the second or third trimester of pregnancy—when the fetal colon has developed—increases risk of offspring obesity. Given the evidence that antibiotics alter microbial communities in the mother and can enter fetal circulation via the placenta, it is plausible that antibiotic use in mid-to-late pregnancy may disrupt seeding of the offspring’s intestinal microbiome by way of the placenta before birth, and/or the mother’s vagina, feces, skin or breast milk during and after birth.

In response to these literature gaps, we examined the hypotheses that CS and antibiotics taken in second or third trimester of pregnancy are independently associated with higher childhood obesity risk and levels of adiposity after controlling for important confounders, including maternal pre-gravid BMI, birth weight, and breastfeeding in the first year of life.
METHODS

Independent variables

Data are from participants in the Columbia Center for Children’s Environmental Health (CCCEH) Mothers and Newborns Study in Northern Manhattan and the South Bronx in New York—a previously described longitudinal birth cohort of mother-offspring dyads. Healthy, nonsmoking, pregnant women (n=727) were recruited from prenatal clinics at New York-Presbyterian Hospital and Harlem Hospital Center between 1998 and 2006. The cohort was restricted to women aged 18-35 years who self-identified as either African-American or Dominican and had resided in the study area for at least 1 year. Women were excluded from the study if their first prenatal visit was after 20 weeks gestation or if they self-reported diabetes, hypertension, known HIV, or use of illicit drugs or cigarettes during pregnancy.

Measures

Prenatal antibiotic use. A questionnaire, administered to each woman in her home by a bilingual interviewer during the third trimester of pregnancy, elicited information on the timing (first, second, and/or third trimester) and duration (in days) of antibiotic use during pregnancy. Prenatal antibiotic use was determined if a mother marked “yes” to antibiotic use during pregnancy, and indicated a duration (days) greater than zero during the trimester of interest. It did not include intrapartum antibiotics.

Mode of delivery. Information on mode of delivery (VD, elective CS, non-elective CS) was abstracted from the mothers’ and infants’ medical records by research staff following delivery. As the effect sizes for elective and non-elective CS were similar (see Results), we combined these two types of CS into one exposure group.
Body mass and composition. At age 7 years, weight (to the nearest 0.1 kg) and body composition were measured using a Tanita scale (model BC-418; Tanita Corporation of America, Arlington Heights, Illinois) while the child wore light clothes and no shoes. The Tanita scale calculated percentage of body fat (%fat), fat mass, and lean mass using bioimpedance formulas validated in children as young as 7 years. Height (to the nearest 0.1 cm) was obtained using a Detecto Cardinal 750 digital scale/stadiometer (Cardinal Scale Manufacturing Company, Webb City, Missouri) until January 2010, and thereafter with the Holtain-Harpenden Wall Mounted Stadiometer Counter 602VR (Holtain Limited, Crymych, UK). Waist circumference was measured halfway between the iliac crest and the lowest rib to the nearest 0.5 cm using non-stretchable tape.

Potential confounders. The questionnaire administered during pregnancy collected information on demographics, history of passive smoke exposure, educational and income levels, receipt of public assistance during pregnancy, maternal height and pre-gravid weight, and number of previous live births. Infant sex, birth weight, and whether the mother was diagnosed with diabetes or hypertension during pregnancy were determined from medical records, and breastfeeding was determined by questionnaire during follow-up.

Statistical analysis

Of the 727 mother-child dyads, we excluded those missing information for delivery mode (23), maternal receipt of public assistance (5), maternal pre-gravid BMI (14), birth weight (50), and gestational age (2). We also excluded those (22) born prematurely (i.e., <37 weeks) to rule out premature birth as a confounding factor. Of the 611 remaining, we successfully followed-up
and measured body mass index (BMI) in 436 children (71.4%) at 7 years of age. Children’s BMI $z$ scores and percentiles were calculated using the SAS Macro provided by the Centers for Disease Control and Prevention. Children were classified as obese if their BMI percentile was greater than or equal to the 95% percentile.

Binomial regression models with robust variance were used to determine if relative risks (RRs) of obesity were higher for children whose mothers had CS (compared to vaginal delivery), or for those whose mothers used antibiotics (compared to not having used antibiotics) in second or third trimester. General linear regression models were used to examine mode of delivery and gestational antibiotics in relation to continuous BMI $z$ score units, % body fat, and waist circumference at 7 years of age. A separate linear regression analysis was conducted to examine whether first trimester antibiotics were associated with BMI $z$ score units. To determine whether gestational antibiotics and CS had compounding impacts, we further compared multivariable adjusted means for BMI $z$ score units across 4 groups: VD + no second or third trimester antibiotics (referent), VD + second or third trimester antibiotics, CS + no second or third trimester antibiotics, and CS + second or third trimester antibiotics (Figure 1).

We assessed confounding by variables previously found to be associated with either exposure (delivery mode or prenatal antibiotics), associated with the outcome (BMI), and that were not considered to be in the causal pathway between the exposure and the outcome. Our core multivariable model included adjustment for child’s sex and birth weight (tertiles), and mother’s age (tertiles), ethnicity (African American/Dominican), pre-gravid BMI (tertiles), and (as a proxy of socioeconomic status) receipt of public assistance during pregnancy (yes/no). In a second model (model 2) we controlled for breastfeeding (yes/no) in the first year to determine whether this might mitigate the observed associations. In a sensitivity analysis we further
excluded 25 children born to mothers who were diagnosed with gestational diabetes or preeclampsia. We also considered models that were additionally adjusted for family income, maternal educational achievement at pregnancy, parity, and mother’s report of a smoker living in the home during pregnancy.

Incomplete follow-up. We conducted sensitivity analyses using inverse probability weights for successful follow-up to assess potential bias due to incomplete follow-up on effect estimates. In estimating the weights we included all variables in our final model that estimated BMI z score, and household income, mother’s satisfaction with living conditions, mother’s years of school completed at time of pregnancy, mother’s neighborhood linguistic isolation, and neighborhood socioeconomic status (poverty rate and median household income) measured using 2000 US Census block group data aggregated to the 1-km radial neighborhood buffers around the home.

All study procedures used at enrollment and at 7 years were approved by the Columbia University Institutional Review Board. Informed consent was obtained from all participating women, and assent was provided by the children at age 7 years.

RESULTS

Table 1 shows the mean (SD) for continuous or n (%) for categorical representation of baseline characteristics for the overall analytic sample, and for the sample according to second or third trimester antibiotic exposure and delivery mode. Of the 436 children included, 70 (16%) had mothers who used antibiotics in the second or trimester, and 99 (22%) were born by CS (46% of which were elective CS). Although not statistically significant, children with mothers
who used antibiotics in the second or third trimester were more likely to be female ($p = 0.17$), have Dominican ethnicity ($p = 0.10$), and have mothers with greater than high-school education ($p = 0.15$). These children did not differ appreciably (all $p \geq 0.25$) by delivery mode, gestational age, birth weight, having been breast fed in first year, nor by mother’s age, parity, receipt of public assistance during pregnancy, or pre-gravid BMI. Children born by CS were less likely to be male ($p = 0.04$) and Dominican ($p = 0.03$) than their VD counterparts. CS children were also marginally more likely to have older mothers ($p = 0.07$) with a previous birth ($p = 0.15$). These groups did not appear to differ on gestational age, birth weight, having been breast fed, nor mother’s use of second or third trimester antibiotics, pre-gravid BMI, educational achievement, or receipt of public assistance in pregnancy (all $p \geq 0.25$).

In Table 2 we present relative risks (RRs) and 95% confidence intervals (CIs) for obesity at age 7. Children exposed to antibiotics in second or third trimester had 84% (33-154%) higher risk of obesity, after full covariate adjustment. Independent of gestational antibiotics, and all other covariates, children born by CS had 46% (8-98%) higher risk of obesity compared to those born vaginally. The association was not significantly different for elective (RR=1.53; 95% CI: 1.04, 2.25) vs. non-elective CS (RR=1.41; 95% CI: 0.97, 2.05) compared to vaginal deliveries. Furthermore, both exposures (second or third trimester antibiotics and CS) remained significantly associated with higher risk of childhood obesity in analyses that excluded children born to mothers diagnosed with gestational diabetes and preeclampsia.

In Table 3 we present findings from multivariable linear regression analyses using continuous BMI z score units, waist circumference, and % body fat measured at 7 years of age that are consistent with the RRs for these exposures in relation to obesity. Adding family income, mother’s educational achievement at pregnancy, parity, or mother’s report of a smoker in the
home did not alter our $\beta$ coefficient for BMI $z$ score by $>$10%, and to maintain a parsimonious model these variables were not included in the final analyses.\textsuperscript{26}

In linear regression analyses (not shown in tables) adjusted for the same covariates above, we found no evidence that first trimester antibiotics were associated with childhood BMI (difference in mean BMI $z$ score units between first trimester antibiotics and no antibiotics= 0.05; 95% CI: -0.41, 0.52). In assessing the independent and joint effect of delivery mode and prenatal antibiotics (Figure 1), we found that associations for delivery mode and second or third trimester antibiotic exposure were similar when stratified by each other. Thus, there was little evidence of interaction on the additive scale. Weighting the data by the inverse probability of follow-up and complete data collection at 7 years did not appreciably change the size of the estimated effect of second or third trimester antibiotics or C-section on BMI $z$ score. The covariate-adjusted weighted $\beta$ coefficient for exposure to antibiotics in the second or third trimester vs. no exposure in the second or third trimester was 0.42 BMI $z$ score units (95% CI: 0.15, 0.68), and the covariate-adjusted weighted $\beta$ coefficient for the C-section delivered children was 0.23 BMI $z$ score units (95% CI: 0.01, 0.46).

**DISCUSSION**

In the present cohort study of 436 mother-child dyads followed up for 7 years from birth of the child, we found that children born to mothers who self-reported taking antibiotics in the second or third trimester of pregnancy had 84% higher risk of obesity at age 7 years compared to children whose mothers did not report antibiotic exposure during this time. Moreover,
independent of prenatal antibiotics, and other confounders, children born via CS had 46% higher obesity risk than VD children.

While previous studies have found that antibiotics administered early in life are associated with increased risk of obesity in childhood,\textsuperscript{7,8} to the best of our knowledge, ours is the first study to report that antibiotic exposure in the second or third trimester of pregnancy may be associated with higher risk of childhood obesity, and greater central adiposity and percent body fat. To determine whether these associations were explained by neonatal exposure to an antibiotic-altered maternal microbiota at birth, we stratified the percent body fat analyses by delivery mode. In these analyses we found a similar magnitude of association, indicating that altered maternal-fetal exchange of vaginal and/or fecal microbiota at birth does not explain the prenatal antibiotics-adiposity link. Rather, it may be due to immunologic and/or metabolic programming occurring before birth.

Until recently, it was believed the fetus and the intrauterine environments were sterile, and, thus, that the intestinal microbiome development began at birth after the newborn’s first microbial inoculum in the vaginal canal or, if delivered CS, contact with skin microbiota.\textsuperscript{2,27,28} However, studies have found evidence of microbes in amniotic fluid,\textsuperscript{29} umbilical cord blood,\textsuperscript{30} meconium,\textsuperscript{31} and placenta\textsuperscript{32,33} and fetal membranes.\textsuperscript{34} These findings, and a recent paper reporting the existence of a distinct human placental microbiome,\textsuperscript{16} call into question the notion of \textit{in utero} sterility. Moreover, they allow for the hypothesis that maternal-fetal exchange of commensal microbiota may occur prior to birth, through exchange of placental bacteria, thereby seeding the intestinal microbiome of the fetus. In this light, antibiotics taken in pregnancy, which can cross the placenta and enter the fetal circulation,\textsuperscript{15} might disrupt normal colonization of the developing intestinal microbiome \textit{in utero} similar to their effect after birth.\textsuperscript{3}
A role for antibiotics in prenatal bacterial ecology has been indicated in other immunological diseases known to be associated with the intestinal microbiome. The Copenhagen Prospective Study on Asthma in Childhood reported an increased risk of asthma exacerbation in children (hazard ratio=1.98; 95% CI: 1.08, 3.63) if their mothers had used antibiotics in the third trimester.\textsuperscript{35} This evidence is supported by other observational studies,\textsuperscript{36–38} and mechanistic research\textsuperscript{39} that demonstrates the involvement of gastric T-cell populations. A systematic review\textsuperscript{40} also found that early-life, including prenatal, exposure to antibiotics was linked to greater risk of eczema in childhood.

Antibiotics taken during pregnancy have also been associated with low birth weight and methylation of imprinted genes.\textsuperscript{41} In our study, we observed an inverse association between prenatal antibiotics and birth weight, which was not statistically significant ($p = 0.25$), but adjustment for birth weight did not alter associations between antibiotics and measures of childhood adiposity. Needed still is investigation on whether methylation of imprinting regions of adipogenesis promoting genes may be on the causal pathway from prenatal antibiotics to childhood obesity.

Independent of prenatal antibiotics, we found that CS was associated with 46% higher risk for obesity in offspring at 7 years. Our results are largely consistent with recent meta-analyses of studies on this topic. One meta-analysis concluded that CS was associated with 33%, 24%, and 50% greater odds of overweight/obesity in children, adolescents, and adults, respectively.\textsuperscript{6} Another meta-analysis of adult studies found that CS compared to VD was associated with 22% greater odds of obesity.\textsuperscript{5} Others have suggested that confounding by maternal pre-gravid body mass may explain at least some of the observed association.\textsuperscript{42} Yet in our study the association persisted after control for pre-gravid BMI, and other potentially
confounding factors, including birth weight, use of prenatal antibiotics, and having breastfed in the first year of life. It has also been suggested that childhood outcomes differ by whether the CS was elective or emergent, but consistent with a meta-analysis of four studies with data on CS type we did not observe such evidence in our cohort. Thus, our findings provide new evidence in support of the hypothesis that CS independently contributes to offspring development of adiposity.

Mounting evidence suggests the CS-obesity association might be attributed to surgically delivered newborns bypassing the bacterial inoculum of the vaginal canal at birth. A growing body of literature has reported differences in the structure of microbial communities between children delivered by CS and those born vaginally. Dominguez-Bello and colleagues demonstrated that the microbiota (across several body habitats, including the skin, oral, nasopharynx, and feces) of vaginally delivered neonates resembled the vaginal microflora of their own mother, whereas the microbiota of neonates born by CS resembled that of the mother’s skin. Other studies have found that stools of CS delivered children have lower counts of *bifidobacteria* and higher counts of *Clostridium difficile* than VD children. And a longitudinal study found that babies delivered by CS had lower overall bacterial diversity up to the age of two years, and delayed colonization of the gut by *bacteriodetes*, compared to their VD counterparts. Exactly how these differences in richness and diversity of overall and specific bacterial communities relate to accumulation of adipose tissue later in life remains to be elucidated.

The strengths of our study included the prospective design, comprehensive set of covariates, and the direct measurement of weight and body composition measured in childhood by trained research staff. Our cohort study was also restricted to non-smoking mothers, thus
eliminating an important potential source of confounding. Another strength of our study was the use of medical records to ascertain mode of delivery, gestational diabetes, and preeclampsia.

Our study has some key limitations. Prenatal use of antibiotics data—including timing and duration—were based on maternal interview. While misclassification of our exposure was inevitable, we do not believe our results were influenced by maternal recall bias, because antibiotic use was self-reported (in the third trimester of pregnancy) before any adverse pregnancy outcomes would have occurred. Another limitation was the lack of information on antibiotic type, dose, and route of administration during pregnancy. We also did not have information about specific medical indications for prescription of antibiotics or data on the severity of the given infections. Thus, it is possible that the maternal infection for which the antibiotics were administered was responsible for the association between prenatal antibiotic use and offspring obesity. There was also substantial drop out in our study, which could potentially bias the results. We addressed potential loss-to-follow-up bias through additional analyses that incorporated inverse-probability weights for successful follow up; the findings from these analyses were not appreciably different from non-weighted models. Nevertheless, inverse probability weighting is only as valid as the predictive model for loss to follow up. Thus, we cannot rule out residual bias from unmeasured factors that influence drop out, and that themselves were associated with exposure and child anthropometric outcomes. Finally, because we did not have data on infant antibiotic use in our cohort, we cannot exclude the possibility that prenatal antibiotic exposure is acting as a proxy for the child having been treated with antibiotics during infancy, and that infant antibiotic usage, not prenatal antibiotic exposure, is causally associated with childhood BMI. However, to the best of our knowledge, there is no literature to
support the supposition that maternal prenatal antibiotics use is strongly associated with the child
being exposed to antibiotics during infancy.

Because of the considerable limitations for, and the novelty of, our findings on prenatal
antibiotics and childhood obesity, we urge replication in other prospective cohort studies before
any conclusions or clinical implications are drawn. Ideally, these studies should be prospective in
nature, and extract information on prenatal antibiotic use from medical records, including the
indication for the prescription, the types of prenatal antibiotics (e.g., broad- vs. narrow-spectrum)
and their mode of administration.

In summary, children born by CS were at higher risk of obesity in childhood, as were
children born to mothers who self-reported taking antibiotics in the second or third trimester of
pregnancy. These exposures were independent of each and other potential confounders,
including maternal pre-gravid BMI, birth weight, and breastfeeding. If other observational
studies can replicate our findings on gestational antibiotics and childhood obesity, then a logical
next step to determine whether this association is causal might be to follow-up children whose
mothers participated in randomized controlled trials of antibiotics during pregnancy. Prospective
observational studies from conception through childhood, with serial measurement of microbial
communities, body weight and body composition, and a comprehensive set of potential
confounding factors are needed to further evaluate relationships between CS and childhood
obesity. A better understanding of how mode of delivery and early-life antibiotics affect our
microbiome may pave new avenues toward the prevention of obesity and related diseases.
Acknowledgments

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Contribution statement

NTM designed the analytic strategy, undertook analyses, interpreted results, and wrote, reviewed and edited the manuscript. RW, LH, MGD, EW, AH, FP, and AR contributed to the study design, results interpretation, and manuscript revision. All authors provided final approval of the manuscript.
References


Figure legend:

Figure 1. Multivariable Adjusted BMI Z Score Means (and Confidence Intervals) for 2nd or 3rd Trimester Antibiotic Use According to Vaginal Delivery (VD) and Cesarean Section (CS)
<table>
<thead>
<tr>
<th>Variables</th>
<th>Total Sample n = 436 (%)</th>
<th>2nd or 3rd Trimester Antibiotics</th>
<th>Delivery Mode</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No n = 366 (%)</td>
<td>Yes n = 70 (%)</td>
<td>Vaginal n = 337 (%)</td>
</tr>
<tr>
<td>Children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boys</td>
<td>47.5</td>
<td>48.9</td>
<td>40.0</td>
</tr>
<tr>
<td>Dominican</td>
<td>39.7</td>
<td>38.0</td>
<td>48.6</td>
</tr>
<tr>
<td>2nd or 3rd Trimester Antibiotics</td>
<td>16.1</td>
<td>-</td>
<td>15.7</td>
</tr>
<tr>
<td>C-section</td>
<td>22.7</td>
<td>22.4</td>
<td>24.3</td>
</tr>
<tr>
<td>Gestational age, weeks, mean (SD)</td>
<td>39.5 (1.2)</td>
<td>39.5 (1.2)</td>
<td>39.3 (1.1)</td>
</tr>
<tr>
<td>Birth weight, g, mean (SD)</td>
<td>3418.5 (473.4)</td>
<td>3429.9 (481.3)</td>
<td>3358.6 (428.0)</td>
</tr>
<tr>
<td>Ever breastfed</td>
<td>74.7</td>
<td>74.7</td>
<td>74.3</td>
</tr>
<tr>
<td>Mothers</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of mother at birth (years)</td>
<td>25.5 (4.9)</td>
<td>25.5 (4.9)</td>
<td>25.2 (4.9)</td>
</tr>
<tr>
<td>mean (SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous births</td>
<td>24.8</td>
<td>24.7</td>
<td>25.7</td>
</tr>
<tr>
<td>0</td>
<td>75.2</td>
<td>75.3</td>
<td>74.3</td>
</tr>
<tr>
<td>Public assistance in pregnancy</td>
<td>57.3</td>
<td>57.1</td>
<td>59.0</td>
</tr>
<tr>
<td>Pre-gravid BMI kg/m², mean (SD)</td>
<td>25.8 (6.0)</td>
<td>25.8 (6.0)</td>
<td>26.0 (6.0)</td>
</tr>
</tbody>
</table>

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Table 2. Relative Risks (and 95% Confidence Intervals) for Childhood Obesity* by Prenatal Exposure to Second or Third Trimester Antibiotics and Mode of Delivery: The Mothers and Newborns Study in Northern Manhattan and South Bronx.

<table>
<thead>
<tr>
<th>2\textsuperscript{nd} or 3\textsuperscript{rd} Trimester Antibiotics</th>
<th>Cases/n</th>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 2 + exclusion of children whose mothers had gestational diabetes or preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>85/366</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Yes</td>
<td>25/70</td>
<td>1.83 (1.36, 2.47)</td>
<td>1.84 (1.33, 2.54)</td>
<td>1.77 (1.25, 2.51)</td>
</tr>
</tbody>
</table>

Mode of Delivery

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Model 2</th>
<th>Model 2 + exclusion of children whose mothers had gestational diabetes or preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td>77/337</td>
<td>1 (Reference)</td>
</tr>
<tr>
<td>Cesarean</td>
<td>33/99</td>
<td>1.43 (1.06, 1.92)</td>
</tr>
</tbody>
</table>

All models exclude those missing information on pre-gravid BMI, gestational age, and birth weight, and only include those with gestational age < 37 weeks

*Obesity defined as BMI percentile ≥ 95\textsuperscript{th} percentile

Model 1: adjusted for sex, ethnicity (Dominican/African American), offspring birth weight (tertiles), maternal age (tertiles), maternal pre-gravid BMI (tertiles), receipt of public assistance during pregnancy (y/n), and prenatal antibiotic use or delivery mode

Model 2: additionally adjusted for having breast fed in 1\textsuperscript{st} year (y/n)

Log-binomial models were used to generate RR and 95% CI

N = 411 after exclusion of mothers diagnosed with gestational diabetes and preeclampsia during pregnancy

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Table 3. Difference in Adjusted Means (and 95% CI) for BMI Z Scores, Waist Circumference, and % Body Fat In Childhood (7 years) By Prenatal Antibiotics and Delivery Mode: The Mothers and Newborns Study in Northern Manhattan and South Bronx.

<table>
<thead>
<tr>
<th>2nd or 3rd Trimester Antibiotics</th>
<th>BMI z score</th>
<th>Waist Circumference (cm)</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Yes</td>
<td>0.47 (0.19, 0.74)</td>
<td>3.13 (0.68, 5.59)</td>
<td>1.86 (0.33, 3.39)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Delivery Mode</th>
<th>BMI z score</th>
<th>Waist Circumference (cm)</th>
<th>% Body Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal</td>
<td>Ref.</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>Cesarean</td>
<td>0.22 (-0.02, 0.46)</td>
<td>1.35 (-0.82, 3.50)</td>
<td>1.04 (-0.33, 2.41)</td>
</tr>
</tbody>
</table>

All models exclude those missing information on pre-gravid BMI, gestational age, and birth weight, and only include those with gestational age < 37 weeks. Models adjusted for sex, ethnicity (Dominican/African American), offspring birth weight (tertiles), maternal age (tertiles), maternal pre-gravid BMI (tertiles), maternal receipt of public assistance during pregnancy (y/n), having breast fed in first year (y/n), and either prenatal antibiotic use (y/n) or mode of delivery.

n=396 (67 with and 329 without 2nd or 3rd trimester antibiotics; 90 with and 306 without cesarean section) for % body fat analyses and 391 (65 with and 326 without 2nd or 3rd trimester antibiotics; 91 with and 300 without cesarean section) for waist circumference analyses.
Figure 1. Multivariable Adjusted BMI Z Score Means (and Confidence Intervals) for 2nd or 3rd Trimester Antibiotic Use According to Vaginal Delivery (VD) and Cesarean Section (CS)

* p<0.05 In Pairwise Comparison with VD + No 2nd/3rd Trimester Antibiotics (Referent)