Gastroenterology 2016;151:120–129

Administration of Antibiotics to Children Before Age 2 Years Increases Risk for Childhood Obesity

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BACKGROUND & AIMS: Childhood obesity is increasing and is associated with adult obesity. Antibiotics have been used to promote weight gain in livestock for several decades. Antibiotics are commonly prescribed for children, but it is not clear how exposure to antibiotics early in life affects risk for obesity. We performed a population-based cohort study to assess the association between antibiotic exposure before age 2 years and obesity at age 4 years. **METHODS:** We performed a retrospective cohort study of 21,714 children in The Health Improvement Network—a population-representative dataset of >10 million individuals derived from electronic medical records from 1995 through 2013 in the United Kingdom. Eligible subjects were registered within 3 months of birth with complete follow-up and height and weight were recorded within 12 months of their 4th birthday. Antibiotic exposure was assessed before age 2 years, and classified based on anti-anaerobic activity. The primary outcome was obesity at age 4 years. We performed logistic regression analyses, adjusting for maternal and sibling obesity, maternal diabetes, mode of delivery, socioeconomic status, year and country of birth, and urban dwelling. **RESULTS:** In the cohort, 1306 of the children (6.4%) were obese at 4 years of age. Antibiotic exposure was associated with an increased risk of obesity at 4 years (odds ratio [OR] = 1.21; 95% confidence interval [CI]: 1.07–1.38). ORs increased with repeated exposures: for 1–2 prescriptions, OR = 1.07 (95% CI, 0.91–1.23); for 3–5 prescriptions, OR = 1.41 (95% CI, 1.20–1.65); and for 6 or more prescriptions, OR = 1.47 (95% CI, 1.19–1.82). Antifungal agents were not associated with obesity (OR = 0.81; 95% CI, 0.59–1.11). **CONCLUSIONS:** Administration of 3 or more courses of antibiotics before children reach an age of 2 years is associated with an increased risk of early childhood obesity.

Keywords: THIN; Microbiome; Pediatrics; UK.

Antibiotics have been used to promote weight gain in the agricultural industry for decades.[1,2] This effect has been hypothesized to be mediated via the gut microbiome, with studies demonstrating no effect on weight gain in germ-free animals.[2,3] The impact of antibiotics on animal weight is greater if given earlier in life.[4]

Childhood obesity is strongly associated with the risk for obesity and its complications in adulthood.[5] There are limited data addressing the effect of antibiotics on human weight gain, despite clear evidence of their overuse in pediatric populations.[6–8] Several studies have retrospectively examined antibiotic use by relying on parental recall to assess exposure to antibiotics. A recent UK study assessed antibiotic exposure in the first 2 years of life and body mass, appreciating an increased risk of obesity at 10 and 20 months and a trend toward a sustained impact at 7 years, although this was not statistically significant after adjusting for parental obesity and tobacco use.[8] Duration, dose, antibiotic class, and household factors, such as sibling obesity, were not assessed. A Danish registry examined antibiotic exposure in the first 6 months of life and demonstrated an increased risk of obesity in children of obese mothers, but noted a protective effect in children of mothers who were not obese. Recurrent exposures could not be assessed.[7] An international survey also found an association between parental recall of antibiotic use in the first 12 months of life and subsequent obesity.[9]

Prior retrospective cohort studies using electronic health records have also suggested an association between antibiotics and obesity.[10–12] However, because of the limitations of the available data, substantial uncertainty remains about the importance of the age of exposure, cumulative frequency of exposure, and spectrum of antibiotics that might influence this association. In addition, uncertainty remains about the potential confounding effects of environmental exposures.

In this study, we examined the association between antibiotic use before 2 years of age and obesity in a large population-representative cohort in the United Kingdom

Abbreviations used in this paper: aOR, adjusted odds ratio; BMI, body mass index; CI, confidence interval; EMR, electronic medical record; OR, odds ratio; THIN, The Health Improvement Network; zWFL, z-weight for length score.

Most current article

0016-5085/$36.00
http://dx.doi.org/10.1053/j.gastro.2016.03.006

with complete follow-up from birth to 48 months, allowing for complete prescription information, adjusting for multiple confounders, including sibling and maternal obesity, method of delivery, year of birth, socioeconomic status, and early obesity. We also assessed whether repeated antibiotic exposures and antibiotics with broader anaerobic coverage and, therefore, potentially greater impact on the microbiome, were more strongly associated with obesity.

Methods

Study Design

We performed a retrospective cohort study using data collected prospectively in the scope of routine care from 1995 to 2013 within The Health Improvement Network (THIN). THIN data are derived from general practitioners' electronic medical records (EMR). THIN represents approximately 6% of the UK population,[13] includes information on age, sex, socioeconomic status, medication use, and has been validated for multiple medical diagnoses.[14–18] Height and weight data are recorded during clinical care. Mother–child pairings and siblings can be determined using a unique household code.[19]

Study Population

We identified individuals who registered with a THIN practice within 3 months of birth who had complete follow-up to 48 months. Individuals were required to have a recorded height and weight within 12 months of their fourth birthday. Height and weight must have been recorded within 1 week of each other. To ensure recording completeness, all individuals born before a practice's installation of the Vision EMR were excluded, as were those who had died, were transferred out of a practice, or did not reach their fourth birthday before the end of data recording. Given the potential for increased antibiotic or glucocorticoid exposures, we excluded individuals with diagnostic codes for reactive airway disease or asthma.

Study Outcomes

The primary outcome was obesity at 48 months, defined using BMI *z*-scores calculated from the UK World Health Organization Term growth reference table using both recorded height and weight, as well as age at the date they were recored.[20] BMI can vary significantly based on age and sex among individuals. To account for this variation, we used *z*-scores, which convert BMIs into numerical values that represent how many standard deviations an individual's BMI is away from the transformed age- and sex-specific population mean. *Z*-scores are employed in World Health Organization and Centers for Disease Control and Prevention growth charts. These values are particularly useful when examining extreme percentiles of the distribution, such as >99% or <1%.[21] Obesity was defined as a BMI *z*-score ≥2.37 for males and ≥2.25 for females based on previously published UK population-specific cutoffs.[22] The time point of 4 years was selected, given the previously described association between obesity at this age and obesity in adulthood.[5]

Variables of Interest

Antibiotic exposure was defined as any prescription for a systemic antibiotic occurring from enrollment in the practice (no later than 3 months after birth) to 2 years of age. We assessed the impact of repeated exposures via number of antibiotic prescriptions (none, 1–2, 3–5, or >5 prescriptions). We also assessed timing of the first antibiotic prescription and timing of first exposure to antibiotics (0–6 months, 6–12 months, or 12–24 months), as prior research had demonstrated a stronger association with exposure in the first year of life.[9] Specific classes of antibiotics were categorized as those with anti-anaerobic coverage (penicillins, imidazoles, lincosamides, and tetracyclines) and without (cephalosporins, macrolides, sulfa-containing agents, isoniazid, rifampin, fluoroquinolones, and aminoglycosides). Systemic antifungal agents were assessed as a control.

We assessed those factors previously identified to be associated with obesity as potential confounders.[23] These included calendar year, geographic region, mode of delivery, socioeconomic status, obesity as an infant, maternal diabetes, and presence of obese family members in the household. A multistep algorithm was employed to identify mothers within the THIN database, adapted from previously published methods within the same database[19] (see Supplementary Methods and Results). Mean maternal BMI was calculated from available height and weight data from age 18 to 240 days before the child's birth. A multilevel variable was then generated to indicate if the mother had a mean BMI >30 kg/m^2, <30 kg/m^2, or if we were unable to identify a mother.

Older siblings of the individuals of interest were identified using similar techniques (see Supplementary Methods and Results). As siblings, we included male and female individuals aged ≤18 years in the same household with complete follow-up during the 4 years of follow-up of the index individual's time within THIN. BMI *z*-scores were calculated using the same criteria as the individuals in our primary cohort of interest. If any siblings met the criteria for obesity during the 4-year follow-up period of the individual within our cohort, they were categorized as obese. A multilevel categorical variable was then constructed, similar in structure to the maternal variable.

For each cohort member, we assessed the presence of obesity during the first year of life. To perform this analysis, we identified height and weight data within 14 days of each other within 12 months of their date of birth. Obesity was calculated for this infant data by calculating *z*-weight for length (zWFL) scores using the World Health Organization growth chart, with obesity defined as a *z*-score >2 SDs above the mean (zWFL > 1.96).

We assessed several additional factors that can influence the risk of obesity at age 4 years. Year of birth was measured to assess the potential influence of trends in obesity over time. Postal codes were used to determine whether cohort members lived in an urban vs rural environment and to compute a Townsend score for socioeconomic status. The Townsend score categorizes socioeconomic status on a 5-point scale, with 5 representing "most deprived," and is derived from percentage of households without access to a car, percentage of households not in owner- occupied accommodations, percentage of households in overcrowded accommodations, and the percentage of the economically active population aged 16–74 years who are unemployed (see Supplementary Methods).

Geographic region was categorized as Northern Ireland, Wales, Scotland, or England. Mode of delivery (cesarean or vaginal) was determined when diagnostic codes were present within 6 months after the birthdate of children of interest. In the subset of individuals who had codes for both vaginal delivery and caesarean delivery, delivery was categorized as caesarean. Maternal diabetes was assessed using diagnostic codes.

Analysis

Statistical analyses were conducted using Stata 13 (StataCorp, College Station, TX). BMI *z*-scores were calculated using the *zanthro* package.[20] Univariable analyses were conducted using logistic regression. We included all potential confounding covariates in a fully adjusted model, without employing a predetermined stepwise backwards elimination strategy to avoid inappropriate overfitting or oversimplification.[24] In a sensitivity analysis, we also generated a more parsimonious model using a backwards elimination stepwise strategy to assess the impact on our results (see Supplementary Methods and Results).

Sensitivity Analyses

We performed several sensitivity analyses related to antibiotic exposure. We assessed the impact of repeated antibiotic prescriptions, from 0 to 10 or more prescriptions, as a continuous variable in univariable and multivariable analyses.[25] We analyzed the impact of anti-anaerobic antibiotics, given their presumed greater impact on the predominantly anaerobic gut microbiome, in univariable analyses and in a combined model stratified on whether the agents were or were not anti-anaerobic.[26] We performed a sensitivity analysis examining our classification of antibiotics with anaerobic coverage, classifying only those with definite anaerobic coverage as anaerobic, and also comparing amoxicillin, which has variable anaerobic coverage, with amoxicillin and clavulanic acid, which has more complete coverage (see Supplementary Methods). We identified households with more than one child within our cohort and assessed the impact of antibiotics among these pairs using conditional logistic regression. We repeated our primary analyses limited to those individuals with both identified mothers and siblings to assess any bias introduced by missing data on obesity among family members.

We assessed interaction between the number of prescriptions and age at first prescription, while avoiding collinearity by combining both age of first antibiotic prescription and number of antibiotics during the first 2 years as a 5-level variable: no antibiotics (reference), 1–2 prescriptions with the first prescription in the first 12 months of life, $\geq$3 prescriptions with the first prescription in the first 12 months of life, 1–2 prescriptions with the first prescription between 12 and 24 months of age, and $\geq$3 prescriptions with the first prescription between 12 and 24 months of age (see Supplementary Methods).

We performed analyses examining the effects of alternate definitions of obesity. For this sensitivity analysis, we used a BMI *z*-score cutoff of $\geq$3 for obesity at age 4 years. To assess the impact of categorizing obesity as a dichotomous variable, we performed linear regression to assess the impact of antibiotic exposures on BMI *z*-score at age 4.

To assess for possible selection bias, we examined the association of antibiotic prescriptions and recording of height and weight at age 4 years. To assess for the potential impact of unmeasured confounders, we determined the strength of association between an unmeasured confounder and the outcome required to explain our observed association between antibiotic exposure and obesity.[27] We modeled our unmeasured confounder after existing data on breastfeeding, as we were unable to measure this in our dataset (see Supplementary Methods). Breastfeeding is known to be associated with both a lower risk of infection and obesity. Approximately 80% of mothers in the United Kingdom breastfeed.[28] A recent meta-analyses demonstrated that breastfeeding is associated with a reduced risk of obesity (odds ratio [OR] = 0.78; 95% confidence interval [CI]: 0.74–0.81).[29] Additionally, earlier studies have demonstrated a protective effect of breastfeeding for respiratory tract infections, with an adjusted OR of 0.65.[30] We also performed an analysis examining the potential confounding effect of obesity during the first 6 months of life in a cohort of individuals with recorded height and weight in that time period using zWFL scores.

This study was considered exempt by the University of Pennsylvania Institutional Review Board and approved by THIN's scientific review committee.

Results

Among 533,238 children identified in THIN within 3 months of birth, 253,157 had 4 years of follow-up. 21,714 children with complete follow-up for 4 years met the inclusion and exclusion criteria (Figure 1); 64.1% were prescribed antibiotics before age 2 years. Median time from birth to cohort entry was 37 days (interquartile range, 25–51 days) and 1306 (6.4%) were obese at age 4 years.

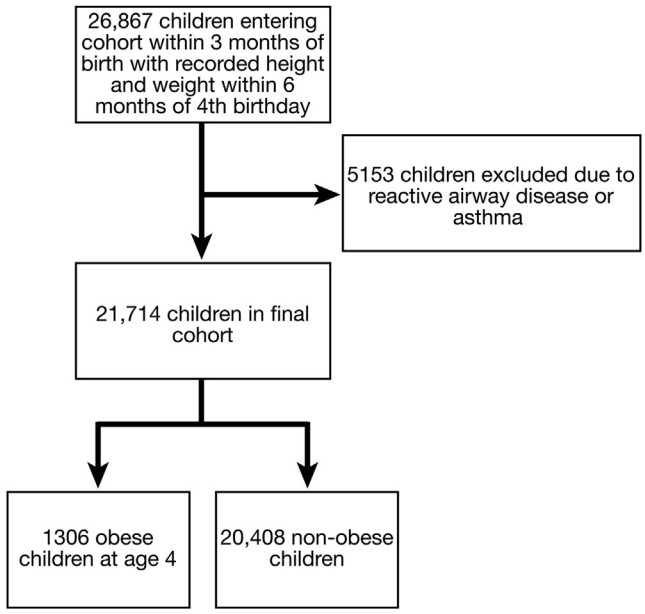

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram. CONSORT diagram of children included in the study, demonstrating those with complete follow-up and height and weight data that were then excluded based on exclusion criteria.

Table 1. Characteristics of Children Within the Cohort, Stratified by the Presence of Obesity at 48 Months

Characteristics	Nonobese (n = 20,408)	Obese (n = 1306)
Born before 2000	4264 (20.9)	219 (16.8)
Born after 2000	16,144 (79.1)	1087 (83.2)
Sex		
Male	10,421 (51.1)	673 (51.5)
Female	9987 (48.9)	633 (48.5)
Area of residence		
Non-urban environment	3360 (16.5)	193 (14.8)
Urban environment	12,319 (60.4)	847 (64.9)
Residence data missing	4729 (23.2)	266 (20.4)
Townsend score[a]		
1	4255 (20.9)	221 (16.9)
2	4024 (19.7)	227 (17.4)
3	4013 (19.6)	239 (18.3)
4	4105 (20.1)	296 (22.7)
5	3172 (15.5)	275 (21.1)
Missing data	839 (4.1)	48 (3.7)
Mother identified	11,715 (57.4)	712 (54.5)
Mother with documented obesity	1308 (6.4)	155 (11.9)
Mother without documented obesity	10,407 (51.0)	557 (42.7)
Mother with diabetes	92 (0.5)	11 (0.8)
Sibling identified	4779 (23.4)	1049 (19.7)
Sibling with documented obesity	325 (1.6)	49 (3.8)
Sibling without documented obesity	4454 (21.8)	208 (15.9)
Mode of delivery identified	5403 (26.5)	336 (25.7)
Caesarean delivery	946 (4.6)	75 (5.7)
Vaginal delivery	4457 (21.8)	261 (20.0)
Antibiotic unexposed	6489 (31.8)	355 (27.2)
Antibiotic exposed	13,919 (68.2)	951 (72.8)
1–2 prescriptions	8269 (40.5)	492 (37.7)
3–5 prescriptions	4149 (20.3)	332 (25.4)
>5 prescriptions	1501 (7.4)	127 (9.7)
Exposure by antibiotic class		
Penicillins	13,244 (64.9)	909 (69.6)
Cephalosporin	992 (4.9)	78 (6.0)
Macrolides	3115 (15.3)	234 (17.9)
Imidazoles	44 (0.2)	2 (0.2)
Isoniazid/rifampin	17 (0.1)	4 (0.3)
Tetracyclines	1 (0.0)	0 (0.0)
Fluoroquinolones	24 (0.1)	1 (0.1)
Aminoglycosides	4 (0.02)	0 (0.0)
Sulfa-based	769 (3.8)	63 (4.8)

[a]Townsend score from least impoverished (1) to greatest level of poverty (5).

Obesity at age 4 years was observed in 5.2% of children without antibiotic exposure and 6.4% of those with antibiotic exposure, with a risk difference of 1.2% (95% CI: 0.1–1.9%). Baseline characteristics of obese and nonobese children are presented in Table 1.

Increasing poverty, urban dwelling, year of birth, maternal and sibling obesity, maternal diabetes, and obesity in the first year of life were associated with an increased risk of obesity at age 4 years (Table 2). The risk of obesity increased with increasing number of courses of antibiotics and with antibiotic use in the first year. After adjusting for all covariates, any antibiotic prescription (adjusted odds ratio [aOR] = 1.21; 95% CI: 1.07–1.38]) and increasing number of antibiotic prescriptions were significantly associated with obesity, specifically for those receiving ≥3 prescriptions (1–2 prescriptions: aOR = 1.07; 95% CI: 0.93–1.23; 3–5 prescriptions: aOR = 1.41; 95% CI: 1.20–1.65; >5 prescriptions: aOR = 1.47; 95% CI: 1.19–1.82). With each increase in antibiotic exposure category, there was an increased risk of obesity (P for trend < .001). Antibiotic use within the first year was also associated with increased risk: (0–6 months: aOR = 1.33; 95% CI: 1.13–1.57; 6–12 months: aOR = 1.27; 95% CI: 1.09–1.47) (Table 3). After assessing for interaction between the number of prescriptions and age at first exposure, repeated exposures before age 2 years had the greatest effect, regardless of whether these exposures started before or after 12 months (before 12 months: aOR = 1.48; 95% CI: 1.27–1.72; after 12 months: aOR = 1.60; 95% CI: 1.22–2.10; Table 4; see Supplementary Methods).

Anti-anaerobic antibiotics were associated with obesity in a dose-dependent manner (1–2 prescriptions: aOR = 1.09; 95% CI: 0.95–1.25; 3–5 prescriptions: aOR = 1.45; 95% CI: 0.91–1.68; >5 prescriptions: aOR = 1.46; 95% CI: 1.09–1.96), while antibiotics without anti-anaerobic activity were not (Table 5). Antifungal agents were not associated with obesity (OR = 0.81; 95% CI: 0.59–1.11).

When assessing the subgroup of individuals for whom we had identified both mothers and siblings (n = 3296), antibiotic exposure was associated with an increased risk of obesity (any exposure: OR = 1.73; 95% CI: 1.16–2.57). When examining families with multiple siblings with discordant outcomes (n = 361), the risk with each antibiotic prescription (aOR = 1.08; 95% CI: 0.90–1.30) and with number of prescriptions (1–2 prescriptions: aOR = 0.72; 95% CI: 0.43–1.24: 3–5 prescriptions: aOR = 1.20; 95% CI: 0.65–2.21; >5 prescriptions: aOR = 1.45; 95% CI: 0.54–4.06) was similar, though not statistically significant.

Sensitivity Analyses

Results in a parsimonious model retaining only covariates that were significant in univariate analyses yielded similar results to our fully adjusted model (see Supplementary Methods and Results). When examining the impact of antibiotic exposures on the BMI z-score at age 4 years, in our fully adjusted model, any antibiotic exposure was associated with a 0.05-point (95% CI: 0.02–0.09) increase in z-score. Each antibiotic prescription was associated with a 0.014-point (95% CI: 0.01–0.02) increase in z-score. When using z-score ≥3 as an alternate definition of obesity, 542 children were classified as obese at age 4 years. In this fully adjusted model, any antibiotic prescription was associated with an increased risk of obesity (aOR = 1.22; 95% CI: 1.01–1.48). Increasing antibiotic use was also associated with an increased risk of obesity (1–2 prescriptions: aOR = 1.06; 95% CI: 0.86–1.32; 3–5 prescriptions: aOR = 1.47; 95% CI: 1.17–1.86; and >5 prescriptions: aOR = 1.39; 95% CI: 1.01–1.92).

Table 2. Univariable and Multivariable Models Assessing the Association of Antibiotic Exposure and All Covariates of Interest With the Risk of Obesity at Age 4 Years

Exposure	Exposed and obese		Univariable analysis, OR (95% CI)	Most fully adjusted model assessing number of prescriptions, OR (95% CI)
	Exposed, n	Obese, n (% of exposed)		
No. of antibiotic prescriptions				
0 (ref)	6844	355 (5.2)	1.00	1.00
1–2	8761	492 (5.6)	1.09 (0.95–1.25)	1.07 (0.93–1.23)
3–5	4481	332 (7.4)	1.46 (1.25–1.71)	1.41 (1.20–1.65)
>5	1628	127 (7.8)	1.55 (1.25–1.91)	1.47 (1.19–1.82)
Maternal obesity				
Nonobese mother identified (ref)	10,964	557 (5.1)	1.00	1.00
No mother identified	9287	594 (6.4)	1.27 (1.13–1.44)	1.25 (1.08–1.43)
Obese mother identified	1463	155 (10.6)	2.21 (1.84–2.67)	1.97 (1.62–2.38)
Maternal diabetes				
Not diabetic (ref)	12,324	701 (5.7)	1.00	1.00
Diabetic mother	103	11 (10.3)	1.98 (1.06–3.72)	1.51 (0.79–2.90)
Sibling obesity				
Siblings identified without obesity (ref)	4662	208 (4.5)	1.00	1.00
No sibling identified	16,678	1049 (6.3)	1.44 (1.23–1.67)	1.42 (1.23–1.68)
Obese sibling identified	374	49 (13.1)	3.23 (2.32–4.50)	2.64 (1.88–3.70)
Country				
England (ref)	16,231	981 (6.0)	1.00	1.00
Northern Ireland	896	48 (5.4)	0.88 (0.65–1.19)	2.08 (1.07–4.11)
Scotland	3401	188 (5.5)	0.91 (0.77–1.07)	2.18 (1.17–4.03)
Wales	1186	89 (7.5)	1.26 (1.01–1.58)	1.09 (0.86–1.37)
Townsend score				
1 (ref)	4476	221 (4.9)	1.00	1.00
2	4251	227 (5.3)	1.08 (0.90–1.31)	1.07 (0.88–1.29)
3	4252	239 (5.6)	1.15 (0.95–1.38)	1.10 (0.91–1.33)
4	4401	296 (6.7)	1.39 (1.16–1.66)	1.30 (1.15–1.63)
5	3447	275 (8.0)	1.67 (1.39–2.00)	1.52 (1.26–1.84)
Missing	887	48 (5.4)	1.10 (0.80–1.52)	2.19 (1.30–3.71)
Urban environment				
Non-urban environment (ref)	3553	193 (5.4)	1.00	1.00
Urban environment	13,166	847 (6.4)	1.20 (1.02–1.41)	1.06 (0.90–1.25)
Missing	4995	266 (5.3)	0.98 (0.81–1.18)	0.39 (0.21–0.76)
Sex				
Male (ref)	11,094	673 (6.1)	1.00	1.00
Female	10,620	633 (6.0)	0.98 (0.88–1.10)	1.03 (0.92–1.15)
Year of birth	—	—	1.04 (1.02–1.06)	1.03 (1.02–1.05)
Obesity in first year				
Nonobese in first year (ref)	8928	471 (5.3)	1.00	1.00
Obese in first year	617	93 (15.1)	3.19 (2.51–4.05)	3.13 (2.46–4.00)
Missing	12,169	742 (6.1)	1.17 (1.04–1.32)	1.14 (1.00–1.29)
Method of delivery				
Vaginal delivery (ref)	4718	261 (5.5)	1.00	1.00
Caesarian section	946	75 (7.4)	1.35 (1.04–1.77)	1.20 (0.92–1.15)
Missing	15,005	970 (6.1)	1.10 (0.96–1.27)	0.97 (0.82–1.14)

When using a more stringent definition of antibiotics with anaerobic coverage, there were 957 individuals exposed to anaerobic antibiotics. Any exposure was associated with an increased risk of obesity at age 4, although this was not statistically significant (OR = 1.21; 95% CI: 0.95–1.55). When comparing those who received no antibiotics to those who had received amoxicillin, amoxicillin and clavulanic acid, or both, the effect estimates were similar for each exposure category (amoxicillin: OR = 1.18; 95% CI: 1.04–1.34; amoxicillin with clavulanic acid: OR = 1.20; 95% CI: 0.70–2.09; both: OR = 1.33; 95% CI: 0.99–1.80) (see Supplementary Methods and Results).

Adjusting for documented obesity within the first 6 months of life did not meaningfully alter the association between repeated antibiotic use and obesity for those who had received ≥3 prescriptions (1–2 prescriptions: aOR = 1.08; 95% CI: 0.93–1.23; 3–5 prescriptions: aOR = 1.41; 95% CI: 1.21–1.65; and >5 prescriptions: aOR = 1.48; 95% CI: 1.19–1.83).

Table 3. Univariable and Fully Adjusted Models Assessing Association Between Number of Antibiotic Prescriptions, Time of First Prescription, and Obesity

Exposure	Exposed, n	Obese, n (% of exposed)	Univariable analysis, OR (95% CI)	Adjusted model assessing no. of prescriptions, OR (95% CI)	Adjusted model assessing age at first prescribed antibiotic, OR (95% CI)
No. of antibiotic prescriptions					
0 (ref)	6844	355 (5.2)	1.00	1.00	—
1–2	8761	492 (5.6)	1.09 (0.95–1.25)	1.07 (0.93–1.23)	—
3–5	4481	332 (7.4)	1.46 (1.25–1.71)	1.41 (1.20–1.65)	—
>5	1628	127 (7.8)	1.55 (1.25–1.91)	1.47 (1.19–1.82)	—
Age at first prescription					
None (ref)	6489	355 (5.2)	1.00	—	1.00
0–6 mo	3837	267 (7.0)	1.37 (1.16–1.61)	—	1.33 (1.13–1.57)
6–12 mo	5851	390 (6.7)	1.31 (1.13–1.51)	—	1.27 (1.09–1.47)
12–24 mo	5182	294 (5.7)	1.10 (0.94–1.29)	—	1.07 (0.91–1.26)

In our analyses of unmeasured confounders and their potential impact on these results, we estimate that failure to adjust for breastfeeding only biased our results for any antibiotic exposure by approximately 2%, with an externally adjusted OR of 1.22 (see Supplementary Methods). If we were to assume that the association between breastfeeding and obesity was underestimated in the meta-analysis by Yan et al,[29] and instead use the strongest effect estimate included in that analysis (OR = 0.29; 95% CI: 0.08–1.05), this would result in 13% bias, with an externally adjusted OR of 1.10. When considering those with ≥3 prescriptions for antibiotics, in whom we observed a greater risk for obesity, the adjusted association between obesity ≥3 antibiotic exposures would be 1.27.[29–31] Within the plausible range of effect estimates, external adjustment for breastfeeding was not able to fully explain the association between antibiotic exposure and obesity observed in this study.

Table 4. Multivariable Model Assessing Interaction Variable of Time of First Antibiotic and Number of Antibiotic Prescriptions With Obesity

Exposure	Multivariable analysis	
	n/N (%)	OR (95% CI)
No antibiotic exposures	345/6590 (5.2)	1.00
1–2 prescriptions with first exposure between 0–12 mo	259/4279 (6.1)	1.18 (0.995–1.39)
3 or more prescriptions with first exposure between 0–12 mo	377/4989 (7.6)	1.48 (1.27–1.72)
1–2 prescriptions with first exposure between 12–24 mo	210/4141 (5.1)	0.96 (0.81–1.15)
3 or more prescriptions with first exposure between 12–24 mo	67/828 (8.1)	1.60 (1.22–2.10)

NOTE. Multivariable model assessing the association of obesity at age 4 y with time of first antibiotic exposure and number of exposures as a combined single interaction variable, adjusted for Townsend quintile (poverty scale), sibling and maternal obesity, and birth before or after 2000. In this model, there is a strong association between exposure to 3 or more antibiotics, regardless of first exposure before or after 12 mo of age.

Receipt of antibiotics was not associated with recording of height and weight among all 253,137 individuals within THIN who had 4 years of follow-up (OR = 1.01; 95% CI 0.98–1.04).

Discussion

With rising rates of childhood obesity worldwide, it is important to identify modifiable contributing factors.[32–35] Antibiotics are prescribed during an estimated 49 million pediatric outpatient visits per year in the United States; the majority are broad-spectrum agents.[6] Between 2006 and 2008, >10 million antibiotic prescriptions were written annually for children without clear indication, despite increased awareness of the societal risks of antibiotic resistance.[36,37] This study identified obesity as one of a growing list of more-tangible risks associated with antibiotic utilization, including dermatologic, allergic, and infectious complications; inflammatory bowel disease; and autoimmune conditions.[38–44] Unlike other potential risks of antibiotic use, the risk of subsequent obesity is likely easily understandable by parents. The results of this study do not imply that antibiotics should not be used when indicated, but rather highlight a reason to avoid antibiotics in the absence of well-established indications. This may be particularly important if the child has been previously treated with antibiotics, as the risk of subsequent obesity was greater in those children who had received ≥3 courses of antibiotics in the first 2 years of life.

These data are supported in both the agriculture industry and murine models. Moore and colleagues[45] first recognized the relationship between streptomycin and weight gain in chick models in 1946. Similar results were appreciated in 1949, in experiments where chickens fed fishmeal supplemented with cobalamin derived from the bacteria *Streptomyces aureofaciens*, which also produced the

Table 5. Impact of Anti-Anaerobic Activity on Association With Obesity

Antibiotic spectrum	1 or more prescriptions (unadjusted)		1 or more prescriptions (adjusted[a] analysis)		Effect per number of prescriptions, adjusted OR[a] (95% CI)			
	n/N (%)	OR (95% CI)	n/N (%)	OR (95% CI)	0	1–2	3–5	>5
No antibiotic exposure	355/6489 (5.5)	1.00	345/6245 (5.5)	1.00	1.00	1.00	1.00	1.00
Any antibiotic exposure	951/13,919 (6.8)	1.24 (1.10–1.42)	931/13,324 (6.9)	1.24 (1.09–1.41)	1.00	1.07 (0.93–1.24)	1.48 (1.27–1.74)	1.52 (1.23–1.89)
Antibiotics with anaerobic coverage	909/13,257 (6.9)	1.24 (1.09–1.39)	872/12,683 (6.9)	1.23 (1.08–1.39)	1.00	1.09 (0.95–1.25)	1.45 (0.91–1.68)	1.46 (1.09–1.96)
Antibiotics without anaerobic coverage	316/4248 (7.4)	1.21 (1.07–1.38)	303/4059 (7.5)	1.21 (1.06–1.38)	1.00	1.09 (0.93–1.27)	1.24 (0.91–1.68)	1.00 (0.63–1.60)

NOTE. Impact of any antibiotic exposure and antibiotic exposure when stratified by anaerobic coverage. Exposure was assessed as any exposure, a continuous exposure with increasing antibiotic prescriptions, and as a categorical variable.
[a]Multivariable models included year of birth, maternal and sibling obesity, maternal diabetes, mode of delivery, country of origin, urban environment, and Townsend score.

antibiotic streptomycin, outgrew chickens receiving fishmeal with a liver-derived B-12 supplement.[4,46] Similar effects have been observed in other livestock with differing antibiotics, and the use of these agents rapidly became commonplace in the agricultural industry.[4] Several laboratory models have demonstrated that increases in weight induced by antibiotics are mediated via the drug's impact on the microbiome, with no effect in germ-free models.[2,47,48] This study is one of the first to demonstrate a similar impact in a human population, while adjusting for multiple factors previously demonstrated to be associated with obesity.

Strengths of the study are the large sample size, near complete capture of lifetime antibiotic exposure, and adjustment for multiple factors, including both maternal and sibling obesity, maternal diabetes, obesity in the first year of life, mode of birth, and socioeconomic status. This design allowed us to disentangle the effects of age at first exposure to antibiotics and the number of courses, identifying repeated antibiotic exposure as the pivotal factor linking antibiotic exposure to childhood obesity. This supports the hypothesis that antibiotics may progressively alter the composition and function of the gut microbiome, thereby predisposing children to obesity as is seen in livestock and animal models.

There are several potential limitations of this research. We did not measure the indication for antibiotic use. However, recent data suggest no causal association between common antibiotic indications and obesity.[10] Children who receive multiple antibiotic prescriptions may differ from those with none or few in ways that relate to future risks of obesity. However, one would expect that in those children with more serious or repeated infections between ages 0 and 2, poor weight gain would be more common than excessive weight gain, thereby biasing our results toward the null. Employing an outpatient medical record, we were unable to capture inpatient medication administration. However, this represents a small minority of prescriptions and would also likely bias the results toward the null. Thus, our design may have slightly underestimated the association of antibiotic exposure and subsequent childhood obesity.

As with any retrospective study, it is possible that selection bias could influence our results. Although growing children in the United Kingdom have routine measurements of their height and weight, these measurements are typically recorded via a paper record known as the Personal Child Health Record, as opposed to in the EMR.[49] Inclusion in the EMR, and subsequently THIN, is at the discretion of the treating physician. Therefore, it is possible that selective recording of this information could influence our findings. However, when examining overall obesity rates in the United Kingdom, observed rates within THIN were only slightly lower than those previously published for the United Kingdom, and demonstrated similar temporal trends within our cohort (data not shown).[50] For selection bias to have influenced the results of this study, recording of height and weight data would have to be associated with both obesity and receipt of antibiotics. As we observed no association between receipt of antibiotics and recording of

height and weight in the overall cohort, selection bias is unlikely to explain the observed associations.

Despite adjusting for many potential confounders associated with obesity, there are a few potential confounders that we were not able to adjust for, including breast feeding, physical activity, and sleep.[23,29,30] However, it is unlikely that these factors fully explain the association between obesity and antibiotic exposure appreciated within our data, given the results of the analyses of unmeasured confounders. The sensitivity analysis using external adjustment demonstrated that our estimate was robust to unmeasured confounders, even if they were strongly protective against future obesity. Furthermore, recent survey data demonstrate that mode of feeding does not usually differ within households,[51] and we appreciated similar effects of repeated antibiotic exposure among sibling pairs discordant for obesity. Although we were able to identify family members in order to adjust for environmental and genetic confounders, we were underpowered to perform a similar analysis examining multiple births only (ie, twins or triplets), as obesity status and antibiotic exposure were nearly identical among these individuals. We also excluded children with asthma or reactive airway disease from our analyses, given the known association between these conditions and obesity and the potential for confounding due to glucocorticoid exposure.[52] Therefore, one should use caution when interpreting the impact of our results in these populations. Further research is required to assess the durability of this response later into adolescence and young adulthood, as well as later-onset obesity.

Lastly, it is possible that our analyses examining antibiotic spectrum coverage could be underpowered and subject to misclassification bias. We appreciated an association between obesity and anti-anaerobic agents, with a dose-dependent response. As the majority of antibiotics prescribed were anti-anaerobic in our primary analyses, it is possible that the lack of association with the use of antibiotics without anti-anaerobic coverage was due in part to smaller numbers. In our sensitivity analysis, amoxicillin, an agent with minimal anti-anaerobic activity, was significantly associated with obesity with an OR comparable with that for amoxicillin with clavulanic acid. Although our analysis of amoxicillin with clavulanic acid did not reach statistical significance, this may be due to limited numbers of individuals with this exposure in comparison with amoxicillin alone (see Supplementary Methods and Results). As such, repeated courses of antibiotic exposure might be a more important risk factor than spectrum of activity. Further research is required to determine whether specific classes of antibiotics are more strongly associated with subsequent obesity.

In summary, we have demonstrated that exposure to antibiotics in the first 2 years of life is associated with an approximately 1.2% absolute and 25% relative increase in the risk of early childhood obesity. This relationship is strongest when considering repeat exposures, particularly with 3 or more courses. While early antibiotic use has been associated with a number of rare long-term health consequences, these data link antibiotics to one of the most important and growing public health problems worldwide.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2016.03.006.

References

1. Jukes TH. Antibiotics in animal feeds and animal production. Bioscience 1972;22:526–534.
2. Gaskins HR, Collier CT, Anderson DB. Antibiotics as growth promotants: mode of action. Animal Biotechnol 2002;13:29–42.
3. Coates ME, Fuller R, Harrison GF, et al. A comparison of the growth of chicks in the Gustafsson germ-free apparatus and in a conventional environment, with and without dietary supplements of penicillin. Br J Nutr 1963;17:141–150.
4. Lassiter CA. Antibiotics as growth stimulants for dairy cattle: a review. J Dairy Sci 1955;38:1102–1138.
5. Serdula MK, Ivery D, Coates RJ, et al. Do obese children become obese adults? A review of the literature. Prev Med 1993;22:167–177.
6. Hersh AL, Shapiro DJ, Pavia AT, et al. Antibiotic prescribing in ambulatory pediatrics in the United States. Pediatrics 2011;128:1053–1061.
7. Ajslev TA, Andersen CS, Gamborg M, et al. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int J Obes (Lond) 2011;35:522–529.
8. Trasande L, Blustein J, Liu M, et al. Infant antibiotic exposures and early-life body mass. Int J Obes (Lond) 2013;37:16–23.
9. Murphy R, Stewart AW, Braithwaite I, et al. Antibiotic treatment during infancy and increased body mass index in boys: an international cross-sectional study. Int J Obes (Lond) 2014;38:1115–1119.
10. Bailey LC, Forrest CB, Zhang P, et al. Association of antibiotics in infancy with early childhood obesity. JAMA Pediatr 2014;168:1063–1069.
11. Azad MB, Bridgman SL, Becker AB, et al. Infant antibiotic exposure and the development of childhood overweight and central adiposity. Int J Obes (Lond) 2014;38:1290–1298.
12. Schwartz BS, Pollak J, Bailey-Davis L, et al. Antibiotic use and childhood body mass index trajectory. Int J Obes (Lond) 2016;40:615–621.
13. Blak BT, Thompson M, Dattani H, et al. Generalisability of The Health Improvement Network (THIN) database: demographics, chronic disease prevalence and mortality rates. Inform Prim Care 2011;19:251–255.
14. Lewis JD, Brensinger C, Bilker WB, et al. Validity and completeness of the General Practice Research Database for studies of inflammatory bowel disease. Pharmacoepidemiol Drug Saf 2002;11:211–218.

15. Ruigomez A, Martin-Merino E, Rodriguez LA. Validation of ischemic cerebrovascular diagnoses in the health improvement network (THIN). Pharmacoepidemiol Drug Saf 2010;19:579–585.
16. Lewis JD, Schinnar R, Bilker WB, et al. Validation studies of the health improvement network (THIN) database for pharmacoepidemiology research. Pharmacoepidemiol Drug Saf 2007;16:393–401.
17. Lo Re V 3rd, Haynes K, Forde KA, et al. Validity of The Health Improvement Network (THIN) for epidemiologic studies of hepatitis C virus infection. Pharmacoepidemiol Drug Saf 2009;18:807–814.
18. Haynes K, Forde KA, Schinnar R, et al. Cancer incidence in The Health Improvement Network. Pharmacoepidemiol Drug Saf 2009;18:730–736.
19. Ban L, Gibson JE, West J, et al. Association between perinatal depression in mothers and the risk of childhood infections in offspring: a population-based cohort study. BMC Public Health 2010;10:799.
20. World Health Organization. WHO Child Growth Standards: Head Circumference-for-Age, Arm Circumference-for-Age, Triceps Skinfold-for-Age and Subscapular Skinfold-for-Age: Methods and Development. Geneva: World Health Organization, 2007.
21. Flegal KM, Ogden CL. Childhood obesity: are we all speaking the same language? Adv Nutr 2011;2:159S–166S.
22. Cole TJ, Bellizzi MC, Flegal KM, et al. Establishing a standard definition for child overweight and obesity worldwide: international survey. BMJ 2000;320(7244):1240–1243.
23. Reilly JJ, Armstrong J, Dorosty AR, et al. Early life risk factors for obesity in childhood: cohort study. BMJ 2005;330(7504):1357.
24. Sainani KL. Multivariate regression: the pitfalls of automated variable selection. PM R 2013;5:791–794.
25. Gamma A. LINCHECK: Stata Module to Graphically Assess the Linearity of a Continuous Variable in a Regression Model. Boston: Boston College Department of Economics, 2005.
26. Guarner F, Malagelada JR. Gut flora in health and disease. Lancet 2003;361(9356):512–519.
27. Orsini N, Bellocco R, Bottai M, et al. A tool for deterministic and probabilistic sensitivity analysis of epidemiologic studies. Stata J 2008;8:29–48.
28. Renfrew M, Pokhrel S, Quigley M. Preventing disease and saving resources: the potential contribution of increasing breastfeeding rates in the UK. Available at: http://www.unicef.org.uk/Documents/Baby_Friendly/Research/Preventing_disease_saving_resources.pdf. Published 2012. Accessed September 24, 2015.
29. Yan J, Liu L, Zhu Y, et al. The association between breastfeeding and childhood obesity: a meta-analysis. BMC Public Health 2014;14:1267.
30. Duijts L, Jaddoe VW, Hofman A, et al. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics 2010;126:e18–e25.
31. van Rossem L, Taveras EM, Gillman MW, et al. Is the association of breastfeeding with child obesity explained by infant weight change? Int J Pediatr Obes 2011;6(2-2):e415–e422.
32. Ogden CL, Carroll MD, Kit BK, et al. Prevalence of obesity and trends in body mass index among US children and adolescents, 1999-2010. JAMA 2012;307:483–490.
33. Lioret S, Touvier M, Dubuisson C, et al. Trends in child overweight rates and energy intake in France from 1999 to 2007: relationships with socioeconomic status. Obesity 2009;17:1092–1100.
34. Orsi CM, Hale DE, Lynch JL. Pediatric obesity epidemiology. Curr Opin Endocrinol Diabetes Obes 2011;18:14–22.
35. Stamatakis E, Wardle J, Cole TJ. Childhood obesity and overweight prevalence trends in England: evidence for growing socioeconomic disparities. Int J Obes (Lond) 2010;34:41–47.
36. Watson RL, Dowell SF, Jayaraman M, et al. Antimicrobial use for pediatric upper respiratory infections: reported practice, actual practice, and parent beliefs. Pediatrics 1999;104:1251–1257.
37. Dowell SF, Marcy SM, Phillips WR, et al. Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections. Pediatrics 1998;101(Suppl 1):163–165.
38. Lessa FC, Gould CV, McDonald LC. Current status of *Clostridium difficile* infection epidemiology. Clin Infect Dis 2012;55(Suppl 2):S65–S70.
39. Kronman MP, Zaoutis TE, Haynes K, et al. Antibiotic exposure and IBD development among children: a population-based cohort study. Pediatrics 2012;130:e794–e803.
40. Goldman JL, Jackson MA, Herigon JC, et al. Trends in adverse reactions to trimethoprim-sulfamethoxazole. Pediatrics 2013;131:e103–e108.
41. Tsakok T, McKeever TM, Yeo L, et al. Does early life exposure to antibiotics increase the risk of eczema? A systematic review. Br J Dermatol 2013;169:983–991.
42. Tahtinen PA, Laine MK, Huovinen P, et al. A placebo-controlled trial of antimicrobial treatment for acute otitis media. N Engl J Med 2011;364:116–126.
43. Hoberman A, Paradise JL, Rockette HE, et al. Treatment of acute otitis media in children under 2 years of age. N Engl J Med 2011;364:105–115.
44. Horton DB, Scott FI, Haynes K, et al. Antibiotic exposure and juvenile idiopathic arthritis: a case-control study. Pediatrics 2015;136:e333–e343.
45. Moore P, Evenson A, Luckey T, et al. Use of sulphasuccidine, streptothricin and streptomycin in nutrition studies with the chick. J Biol Chem 1946;165:437–441.
46. Stokstad EL, Jukes TH, et al. The multiple nature of the animal protein factor. J Biol Chem 1949;180:647–654.
47. Cho I, Yamanishi S, Cox L, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488(7413):621–626.
48. Cox LM, Yamanishi S, Sohn J, et al. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 2014;158:705–721.
49. Royal College of Paediatrics and Child Health. Early years–UK-WHO growth charts and resources. Available at: http://www.rcpch.ac.uk/child-health/research-projects/

uk-who-growth-charts/uk-who-growth-chart-resources-0-4-years/uk-who-0. Published 2015. Accessed January 21, 2016.

50. van Jaarsveld CH, Gulliford MC. Childhood obesity trends from primary care electronic health records in England between 1994 and 2013: population-based cohort study. Arch Dis Child 2015;100:214–219.
51. McAndrew F, Thompson J, Fellows L, et al. Infant Feeding Survey 2010. Information Centre for Health and Social Care. Available at: http://www.hscic.gov.uk/catalogue/PUB08694/Infant-Feeding-Survey-2010-Consolidated-Report.pdf. Accessed May 11, 2016.
52. Black MH, Smith N, Porter AH, et al. Higher prevalence of obesity among children with asthma. Obesity 2012; 20:1041–1047.

Received October 31, 2015. Accepted March 13, 2016.

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Conflicts of interest
The authors disclose no conflicts.

Funding
This research was supported in part by grants K08-DK095951 (Frank I. Scott), K24-DK078228 (James D. Lewis), and K08-DK098272 (David S. Goldberg) from the National Institute of Diabetes and Digestive and Kidney Diseases, as well as grants K23-CA187185 (Ronac Mamtani) from the National Cancer Institute and F32-AR066461 (Daniel B. Horton) from the National Institute of Arthritis and Musculoskeletal and Skin Diseases and by the University of Pennsylvania's Center for Pharmacoepidemiology Research and Training and the Penn-CHOP Microbiome Program.

Supplementary Methods and Results

Derivation of Mothers Within The Health Improvement Network

A multistep algorithm was employed to identify mothers within the THIN database, adapted from a previously published algorithm developed within the same database.[1] All individuals living within the same household within a specific THIN practice are assigned a unique family identifier. For each child included in the study, we identified all female individuals of child-bearing age, defined as >15 years old and <55 years old living in the same household at the time of the child's birth. If there was only one eligible candidate, this was defined as the likely mother. If multiple potential maternal candidates were identified, we then also assessed for the presence of pregnancy or delivery-related diagnostic codes for each candidate from 1 year before to 30 days after the child's birth. If there was still more than 1 possible maternal candidate, both were removed. Mean maternal BMI was calculated from available height and weight data from age 18 to 240 days before the child's birth. A multilevel variable was then generated to indicate if the mother had a mean BMI >30 kg/m^2, <30 kg/m^2, or if we were unable to ensure we had identified a mother.

Derivation of Siblings Within The Health Improvement Network

Using similar techniques as with maternal identification, we also identified siblings of the patients of interest. To perform this analysis, we identified male and female individuals aged ≤18 years with complete follow-up during the 4 years of follow-up of the index individual's time within THIN. We then identified height and weight data on these individuals, and calculated BMIs using height and weight data within 1 week of each other. The *z*-BMI scores were calculated using the same criteria as the individuals of interest. If at any point the siblings were obese during the 4-year follow-up period, the individual was identified as having been exposed to an obese sibling. A multilevel categorical variable was then constructed, similar in structure to the maternal variable.

Derivation of the Townsend Score Within The Health Improvement Network Database

The Townsend classification was originally defined by Professor Peter Townsend. The score employs census data to generate a deprivation index. The index is derived geographically using 2001 census data in the United Kingdom, although similar methods can be applied when similar census data are available. The index is constructed from the following variables collected in census taking: households without a car, number of overcrowded households, number of households that are not occupied by the owner, and number of unemployed individuals aged 16–74 years.

Each of these variables is divided by the total number of households within the postal code of interest. Unemployment rates and overcrowding percentages are then log-transformed. The subsequent results are then transformed into *z*-scores and summed. Townsend scores within THIN are then assigned in quintiles based on level of deprivation, from 1 to 5, with 5 being the most deprived. However, it is important to note that in this dataset, if there were <20 households in the denominator, a missing value was applied. These quintile values are then reported in the dataset, when available. Postal codes and individual components of the Townsend score are not reported in THIN.

Evaluation of Interaction Between Number of Antibiotic Courses and Age at First Antibiotic Prescription

A multivariable model was initially constructed assessing the relationship between both number of antibiotic prescriptions (1–2, 3–5, or >5) and time of first antibiotic prescription (0–6 months, 6–12 months, or 12–24 months), adjusting for maternal and sibling obesity, Townsend score, and decade of birth. Due to collinearity between the 2 exposure variables, the model could not provide separate estimates for the earliest age of first exposure and the category for the greatest number of prescriptions.

A new variable was then constructed combining both age of first antibiotic prescription and number of antibiotics during the first 2 years as a 5-level variable: no antibiotics (reference group), 1–2 prescriptions with the first prescription in the first 12 months of life, ≥3 prescriptions with the first prescription in the first 12 months of life, 1–2 prescriptions with the first prescription between 12 and 24 months of age, and ≥3 prescriptions with the first prescription between 12 and 24 months of age. When assessed in a multivariable logistic regression model adjusting for maternal and sibling obesity, Townsend score, and decade of birth, we observed a statistically significant association for ≥3 antibiotic prescriptions, regardless of age at the time of the first antibiotic prescription (see Table 5). In contrast, receipt of 1–2 prescriptions was not statistically significant regardless of the age at the time of receipt of the first prescription. These results demonstrate that the number of prescriptions is more strongly associated with risk of obesity than timing of first prescription.

Sensitivity Analysis Examining the Classification of Anaerobic Coverage With Individual Antibiotics

In our primary analyses, anti-anaerobic activity was categorized in terms of class of antibiotics. However, the spectrum of anti-anaerobic activity varies among the members of some classes of antibiotics. For example, penicillin and amoxicillin have minimal anti-anaerobic activity in comparison with other members of the same class (eg, methicillin or amoxicillin plus clavulanic acid). Therefore, we performed a sensitivity analysis restricting the definition of anaerobic antibiotics to only those with more robust coverage, removing the penicillins (with the exception of cloxacillin, methicillin, and amoxicillin with clavulanic acid), as well as tetracyclines from the previously defined anaerobic group. As the majority of prescriptions

were for amoxicillin and penicillin, this reduced the number of individuals exposed to this new classification of anaerobic antibiotics. In this modified analysis, there were 957 individuals with an exposure to this new classification of anti-anaerobic antibiotics, of which 68 were obese. Any exposure to a strictly defined anti-anaerobic antibiotic was associated with a similar effect estimate as seen in our primary analyses, though this was not statistically significant (OR = 1.21; 95% CI: 0.95–1.55).

To explore the relative impact of penicillins with and without anaerobic coverage, we also assessed a multilevel variable using our full cohort, adjusting for other antibiotic use. This new covariate assessed no exposure compared with amoxicillin and clavulanic acid only, amoxicillin only, or to both, adjusted for all other antibiotic exposures (Supplementary Table 1).

Based on both of these analyses, it appears that while anti-anaerobic agents can contribute to the development of obesity, the number of exposures to antibiotics is a more important risk factor for obesity.

Sensitivity Analysis of an Unmeasured Confounder's Influence on the Association Between Antibiotics and Obesity

We were not able to measure breastfeeding, a potential confounder that may be associated with both antibiotic exposure and obesity. Breastfeeding rates in the general population within the United Kingdom are estimated to be approximately 80%.[2] Earlier literature has demonstrated a protective effect of breastfeeding on upper respiratory tract infections, with an adjusted OR of 0.65.[3] In addition, a recently published meta-analysis demonstrated an association between breastfeeding and a reduced risk of obesity (OR = 0.78).[4] Based on these findings, we performed a sensitivity analysis examining what impact unmeasured confounding by a variable such as breastfeeding can have on our estimated association between antibiotics and obesity.

To perform these analyses, we employed methods for external adjustment as previously described by Greenland.[5] In order to estimate the impact of an unmeasured confounder, we require: (a) the antibiotic-specific association of breastfeeding with obesity risk; (b) the antibiotic-specific prevalence of breastfeeding in nonobese individuals (controls); and (c) the prevalence of antibiotic exposure among nonobese individuals. While we can measure (c) within our cohort of individuals, we are required to estimate parameters (a) and (b). We initially measured rates of antibiotic exposure among our cohort, stratified by obesity (Supplementary Table 2).

From this cohort, we can calculate an $OR_{DXunadj.} = 1.24$ (95% CI: 1.09–1.40), which is similar to our fully adjusted OR in our regression model of OR = 1.21 (95% CI: 1.06–1.38). To calculate an OR while adjusting for breastfeeding, we can stratify our cohort based on exposure to breastfeeding (Supplementary Table 3). We can calculate the values for the individual cells and OR_{DXadj} using our available data and estimates of the impact of breastfeeding on obesity and prevalence of obesity.

Using the estimated prevalence of breastfeeding in those exposed and unexposed to antibiotics, we can calculate those cells involving our nonobese individuals (B_{11} and B_{01}). We know the mean rate of breastfeeding in the United Kingdom is 80%.[2] We will assume that the breastfeeding rate in those individuals who did not receive antibiotics is equivalent to the baseline population rate, or $P_{Z0} = 0.80$. To calculate the baseline rate of breastfeeding among those who did receive antibiotics (P_{Z1}), we will initially assume that breastfeeding is completely protective against antibiotic utilization, and that differential rates of breastfeeding can completely explain differences in antibiotic usage rates. This is a conservative estimate, as it is unlikely that breastfeeding alone completely explains the heterogeneity among antibiotic and nonantibiotic users.

If we had a cohort of 100 individuals who did not receive antibiotics, then among those, 80 would have been breastfed ($P_{Z0} = 0.80$). We can then construct a 2 × 2 table and calculate the estimated rate of breastfeeding in those who did not receive antibiotics using the previously published OR of the protective effect of antibiotics against infection (OR = 0.65)[3] (Supplementary Table 4).

We estimate that among those receiving antibiotics, the prevalence of the confounder breastfeeding was 0.722. Given the uncertainty in this estimate, we can later perform sensitivity analyses on these prevalences as well. We can then use these prevalences to calculate $B_{11} = P_{Z1}B_{1+}$ and $B_{01} = P_{Z0}B_{0+}$:

$B_{11} = (0.7222)(13{,}257) = 9574.2$

$B_{01} = (0.80)(7151) = 5720.8$

We can then calculate A_{11} and A_{01} as described by Greenland[5]:

$A_{11} = OR_{DZ}\ A_{1+}\ B_{11}/\ (OR_{DZ}B_{11} + B_{01}\text{-}B_{11}) = 608.78$

$A_{01} = OR_{DZ}\ A_{0+}\ B_{01}/\ (OR_{DZ}B_{01} + B_{01}\text{-}B_{01}) = 300.64$

Where OR_{DZ} is the OR of the association between breastfeeding and obesity (0.78). We can then solve for $OR_{DXadj} = A_{11}\ B_{01}\ /\ A_{01}\ B_{11} = 1.21$. Therefore, our original estimate of the association between antibiotics and obesity was biased by approximately 2% by the unmeasured impact of breastfeeding, using these real world estimates. We can then vary the prevalence of breastfeeding in those antibiotic exposed, and the OR of the protective effect of breastfeeding for obesity (OR_{DZ}) to assess how strong the effect would need to be to bias our results to the null (Supplementary Table 5).

Based on these results, breastfeeding would be required to be both substantially less common in those who received antibiotics, and breastfeeding would need to have a considerably stronger protective effect against infection than what has been previously reported in the literature, for our estimate to be negated by bias introduced by this confounder.

Parsimonious Model

We created a more parsimonious model using a backwards elimination strategy, retaining only those covariates that remained statistically significant or modified our effect estimates by >10%. This did not significantly modify our results. After adjusting for maternal obesity, sibling obesity, Townsend score, and decade of birth, any antibiotic exposure (aOR = 1.24; 95% CI: 1.09–1.41) and repeated exposures

were associated with an increased rate of obesity at age 4 years (1–2 prescriptions: OR = 1.07; 95% CI: 0.93–1.24; 3–5 prescriptions: OR = 1.48; 95% CI: 1.27–1.74); and >5 prescriptions: OR = 1.52; 95% CI: 1.23–1.89).

References

1. Ban L, Gibson JE, West J, Tata LJ. Association between perinatal depression in mothers and the risk of childhood infections in offspring: a population-based cohort study. BMC Public Health 2010;10:799.
2. Renfrew M, Pokhrel S, Quigley M. Preventing disease and saving resources: the potential contribution of increasing breastfeeding rates in the UK. Available at: http://www.unicef.org.uk/Documents/Baby_Friendly/Research/Preventing_disease_saving_resources.pdf. Published 2012. Accessed September 24, 2015.
3. Duijts L, Jaddoe VW, Hofman A, et al. Prolonged and exclusive breastfeeding reduces the risk of infectious diseases in infancy. Pediatrics 2010;126:e18–e25.
4. Yan J, Liu L, Zhu Y, et al. The association between breastfeeding and childhood obesity: a meta-analysis. BMC Public Health 2014;14:1267.
5. Greenland S. Basic methods for sensitivity analysis of biases. Int J Epidemiol 1996;25:1107–1116.

Supplementary Table 1. Association Between Amoxicillin Plus Clavulanic Acid vs Amoxicillin Alone With Obesity

Exposure	No. exposed	No. obese	Adjusted OR	95% CI
No exposure	8304	472	Ref	Ref
Amoxicillin and clavulanic acid only	199	14	1.20	0.70–2.09
Amoxicillin without clavulanic acid	11,253	768	1.18	1.04–1.34
Exposure to both	652	52	1.33	0.99–1.80

Supplementary Table 2. Cohort Stratified by Antibiotic Exposure and Obesity at Age 4

	Any antibiotic = 1	Any antibiotic = 0
Obese = 1	$A_{1+} = 909$	$A_{0+} = 397$
Obese = 0	$B_{1+} = 13{,}257$	$B_{0+} = 7151$

Supplementary Table 3. Stratification by Obesity, Antibiotic Exposure, and an Unmeasured Confounder

	Breastfeeding = 1		Breastfeeding = 0	
	Any antibiotic = 1	Any antibiotic = 0	Any antibiotic = 1	Any antibiotic = 0
Obese = 1	A_{11}	A_{01}	A_{1+}-A_{11}	A_{0+}-A_{01}
Obese = 0	B_{11}	B_{01}	B_{1+}-B_{11}	B_{0+}-B_{01}

Using the stratified tables, our OR adjusted for breastfeeding would be:
$OR_{DXadj} = A_{11}\ B_{01}\ /\ A_{01}\ B_{11} = (A_{1+}\text{-}A_{11})(B_{0+}\text{-}B_{01})/(A_{0+}\text{-}A_{01})(B_{1+}\text{-}B_{11})$.

Supplementary Table 4. Estimated Rate of an Unmeasured Confounder Among Antibiotic Exposed

	Breastfeeding = 0	Breastfeeding = 1
–Antibiotics	20	80
+Antibiotics	100 – x	x

OR_{Abx}: 20x / 80 (100 – x) = 0.65.
X = 72.2.

Supplementary Table 5. Estimated Bias Introduced by Unmeasured Confounder

		OR_{DZ}		
P_{Z0}	P_{Z1}	0.78 (% Bias)	0.65 (% Bias)	0.50 (% Bias)
0.80	0.72	1.21 (2)	1.19 (4)	1.16 (7)
0.80	0.65	1.19 (4)	1.15 (7)	1.10 (12)
0.80	0.50	1.14 (8)	1.08 (15)	0.99 (25)
0.80	0.35	1.10 (12)	1.01 (22)	0.90 (37)