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PEDIATRIC ORIGINAL ARTICLE

Infant antibiotic exposure and the development of childhood overweight and central adiposity

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BACKGROUND: Obesity has been associated with disruption of the gut microbiota, which is established during infancy and vulnerable to disruption by antibiotics.
OBJECTIVES: To investigate the association between early-life antibiotic exposure and subsequent development of overweight and central adiposity.
METHODS: Provincial health-care records were linked to clinical and survey data from a Canadian longitudinal birth cohort study. Antibiotic exposure during the first year of life was documented from prescription records. Overweight and central adiposity were determined from anthropometric measurements at ages 9 ($n=616$) and 12 ($n=431$). Associations were determined by multiple logistic regression.
RESULTS: Infants receiving antibiotics in the first year of life were more likely to be overweight later in childhood compared with those who were unexposed (32.4 versus 18.2% at age 12, $P=0.002$). Following adjustment for birth weight, breastfeeding, maternal overweight and other potential confounders, this association persisted in boys (aOR 5.35, 95% confidence interval (CI) 1.94–14.72) but not in girls (aOR 1.13, CI 0.46–2.81). Similar gender-specific associations were found for overweight at age 9 (aOR 2.19, CI 1.06–4.54 for boys; aOR 1.20, CI 0.53–2.70 for girls) and for high central adiposity at age 12 (aOR 2.85, CI 1.24–6.51 for boys; aOR 1.59, CI 0.68–3.68 for girls).
CONCLUSIONS: Among boys, antibiotic exposure during the first year of life was associated with an increased risk of overweight and central adiposity in preadolescence, indicating that antibiotic stewardship is particularly important during infancy. Given the current epidemic of childhood obesity and the high prevalence of infant antibiotic exposure, further studies are necessary to determine the mechanisms underlying this association, to identify the long-term health consequences, and to develop strategies for mitigating these effects when antibiotic exposure cannot be avoided.

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INTRODUCTION

Childhood obesity is among the most serious public health challenges in the world today.[1] Although poor diet and low physical activity are well-known contributors to this international epidemic, accumulating evidence points to early-life origins of obesity, such as low or high birth weight, rapid growth during infancy and maternal nutrition, smoking or stress during pregnancy.[2] Recent findings from a prospective cohort of US children show that a substantial component of childhood obesity is established before school entry, emphasizing the important role of the early postnatal environment.[3] Meanwhile, observations that lean and obese individuals harbor different communities of gut bacteria have inspired the notion that weight gain may be influenced by commensal microbes.[4–9] Antibiotics can radically alter the gut microbiota at any age,[10,11] but this may be especially consequential during early postnatal life when the developing microbiota is particularly vulnerable to disruption by environmental influences.[12,13] It is therefore plausible that infant antibiotic use could be a previously under-appreciated determinant of overweight. Indeed, alongside worldwide increases in obesity, the use of antibiotics has increased substantially in the last half-century.[14]

Despite exhibiting a period of decline in the late 1990s and early 2000s, antibiotic use remains common among children in developed countries, and the use of newer broad-spectrum antibiotics has risen many-fold over the same time period.[15–18] In 2007, the proportion of European infants receiving antibiotic prescriptions in their first year of life ranged from 18% in Switzerland to 55% in Italy.[19] In the UK, antibiotic prescribing to children of all ages has been steadily increasing since 2000.[20]

The growth-promoting effects of antibiotics are well-known in livestock farming, where low-dose treatment is found to improve animal weight gain and feed efficiency.[21] Supplementation is most effective in early life, whereas no growth benefit is seen when antibiotics are given to mature animals.[22] Similarly in mice, early postnatal antibiotic exposure has been shown to increase total fat mass and percent body fat.[23] Recent evidence from two European birth cohorts suggests that a similar association may exist in humans.[24,25] Using data from the Danish National Birth Cohort, Ajslev *et al.*[24] found that early-life antibiotic use increased the risk of overweight at age 7 among children born to normal weight mothers. From the UK Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, Transande *et al.*[25] have reported that antibiotic use in the first 6 months of life was associated with an

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elevated risk of overweight and consistent increases in body mass up to 38 months of age. In addition, new findings from a large international cross-sectional survey indicate that antibiotic exposure during the first year of life is associated with increased childhood body mass index (BMI) in boys aged 5–8 years.[26] These initial human studies have relied on parent report of infant antibiotic use, and have not reported outcomes beyond 8 years of age. Moreover, all three studies documented total adiposity, but not central adiposity, which is considered a better predictor of obesity-related health outcomes in adults and school-age children.[27–30]

Here, we evaluate the association of infant antibiotic use and the development of overweight and central adiposity in a cohort of Canadian children followed to 12 years of age, using documented prescription records to classify antibiotic exposure.

SUBJECTS AND METHODS

Study population

This was a secondary analysis of provincial health-care databases (prescriptions, hospitalizations and physician visits) linked to clinical and survey data in a longitudinal 1995 birth cohort study (known as the Study of Asthma, Genes and the Environment, or SAGE) in Manitoba, Canada. Full details of the SAGE study design and data linkages are reported elsewhere.[31,32] Briefly, in 2002, a one-page survey was mailed to the households of all Manitoba children born in 1995 and still residing in the province at 7 years of age ($N = 13\,980$). A total of 3598 surveys were returned, and a nested case–control study was populated from these data, including 723 children (246 with asthma and 477 controls) whose parents agreed to participate and gave permission to link study data with their child's health-care records. These children were seen for clinical assessments, including anthropometric measurements, at ages 9 (range 8–10) and 12 (range 11–14). The current study population comprised all children with available prescription records and anthropometric data ($N = 616$ of the 723 children from the nested case–control arm of SAGE); prescription records were unavailable for 107 case–control children, most commonly because they were living on First Nations reserves where prescription data are not accurately captured in provincial databases. We have accounted for the overrepresentation of asthmatic children in SAGE by evaluating asthma as a covariate and potential confounder in our multivariate regression models. This study was approved by the Health Research Ethics Board at the University of Manitoba and the Health Information Privacy Committee.

Antibiotic use

As described previously,[31] antibiotic use during the first year of life was determined from provincial prescription records, identified on the basis of the Anatomical Therapeutic Chemical classification system and generic drug names. Our primary exposure variable was 'any antibiotic exposure in the first year of life', regardless of the specific timing, type of antibiotic or number of courses. Antibiotic exposure was further classified according to the number of prescriptions in the first year (zero, one to two, three to four and five or more courses), the timing of first antibiotic exposure (never in the first year, < 3 months, 3–6 months or 6–12 months) and the type of antibiotics received (none, broad-spectrum, narrow-spectrum or both). Penicillin, cloxacillin, cephalexin, cefadroxil and erythromycin were defined as narrow-spectrum antibiotics, and all others fell into the category of broad-spectrum antibiotics.

Child overweight and central adiposity

We employed two direct anthropometric measures of adiposity: total adiposity was measured at ages 9 and 12 according to BMI (weight in kilograms per height in meters^2), and central adiposity was measured at age 12 according to waist circumference (measured at the high point of the iliac crest to the nearest 0.1 cm). As recommended by an Expert Committee endorsed by the American Academy of Pediatrics and the Obesity Society,[33] pediatric overweight was defined as BMI $>$85th percentile according to the US Centres for Disease Control age and sex-standardized growth charts.[34] High central adiposity was defined as waist circumference $>$75th percentile according to published age and sex-standardized reference charts derived from a nationally representative sample of US children;[35] the 75th percentile cut point was selected based on its recommended use in clinical settings for identifying children at risk for type 2 diabetes.[36]

Other covariates

Parents and children completed standardized surveys at each follow-up, reporting on various health and lifestyle factors. Survey-derived covariates included exclusive breastfeeding ≥ 3 months, annual family income and early-life exposure to environmental tobacco smoke (determined from the question: 'In 1995 (child's birth year), did anyone living in your home smoke (cigarettes or tobacco)?'). At age 9, parents reported their child's weekly physical activity, consumption of various foods and daily screen time (computer, video games, television). A modified version of the Youth Healthy Eating Index was administered to children at age 12.[37]

Child asthma was diagnosed by a pediatric allergist at each study visit.[32] Maternal asthma during pregnancy, which has been shown to influence long-term health outcomes in offspring,[38] was documented from provincial health records (at least one physician visit or hospitalization for asthma or one prescription for an asthma drug during the 5 years preceding the child's birth). Maternal overweight (BMI ≥ 25 kg m^{-2}) was determined from actual height and weight (measured at the age 12 follow-up), or if measurements were unavailable, from self-reported height and weight (collected at the age 10 follow-up).

Gender, birth weight, method of delivery, birth order, number of siblings at age 7 and urban (municipality population $>40\,000$) or rural residence location were documented from health-care records. Infections in the first year of life were also identified from health-care records, including physician visits for lower respiratory tract infections (e.g., bronchitis, bronchiolitis and pneumonia), upper respiratory tract infections (e.g., otitis media, pharyngitis and sinusitis) and non-respiratory tract infections (e.g., genitourinary infections, cellulitis and impetigo). Lower respiratory tract infection was considered a proxy indicator for infant wheezing, a potential confounder in this analysis because early-life wheezing is commonly treated with antibiotics,[17,39] and has been independently associated with overweight.[40–42]

Statistical analysis

The likelihood of overweight or high central adiposity at ages 9 or 12 according to antibiotic exposure during the first year of life was determined using multiple logistic regression analysis, conducted with the statistical software package SAS 9.2 (SAS Institute; Cary, NC, USA). Associations (odds ratios) are reported at the 95% level of confidence. Among children receiving antibiotics, we also tested for linear trends according to the number of courses received. Previous reports showed greater effects of antibiotic exposure among boys versus girls,[24,26] and among infants born to normal-weight versus overweight mothers;[24,25]

Table 1. Prevalence of early-life antibiotic exposures and childhood overweight, overall and by gender

	All children N = *616*	*Girls* N = *270*	*Boys* N = *346*	
Early-life antibiotic exposures	n %	n %	n %	P
Any antibiotics in first year	438 (71.1)	180 (66.7)	258 (74.6)	0.03
Number of courses				
None	178 (28.9)	90 (33.3)	88 (25.4)	0.09
1–2	228 (37.0)	94 (34.8)	134 (38.7)	
3–4	115 (18.7)	52 (19.3)	63 (18.2)	
>4	95 (15.4)	34 (12.6)	61 (17.6)	
Type of antibiotics received				
None	178 (28.9)	90 (33.3)	88 (25.4)	0.17
Narrow spectrum only	13 (2.1)	6 (2.2)	7 (2.0)	
Broad spectrum only	354 (57.5)	147 (54.4)	207 (59.8)	
Both	71 (11.5)	27 (10.0)	44 (12.7)	
Age at first antibiotic prescription				
No antibiotics	178 (28.9)	90 (33.3)	88 (25.4)	0.17
<3 months	73 (11.9)	32 (11.9)	41 (11.8)	
3 to <6 months	133 (21.6)	56 (20.7)	77 (22.3)	
6 to <12 months	232 (37.7)	92 (34.1)	140 (40.5)	
Childhood overweight outcomes				
Overweight at age 9 ($N = 616$)	181 (29.4)	73 (27.0)	108 (31.2)	0.26
Overweight at age 12 ($N = 431$)	121 (28.1)	45 (24.7)	76 (30.5)	0.19
High central adiposity at age 12 ($N = 428$)	145 (33.9)	55 (30.6)	90 (36.3)	0.22

Comparisons between genders by χ^2 test.

Table 2. Prevalence and distribution of potential confounding variables according to infant antibiotic exposure and child overweight outcomes

	Overall prevalence N = *616*[a]	*Exposed to antibiotics during first year* N = *616*[a]		*Overweight at age 9* N = *616*[a]		*Overweight at age 12* N = *431*[a]		*High central adiposity at age 12* N = *428*[a]	
	n *(%)*	n *(%)*	P	n *(%)*	P	n *(%)*	P	n *(%)*	P
Gender									
Female	270 (43.8)	180 (66.7)	0.03	73 (27.0)	0.26	45 (24.7)	0.19	55 (30.6)	0.22
Male	346 (56.2)	258 (74.6)		108 (31.2)		76 (30.5)		90 (36.3)	
Mode of delivery (N = *614*)									
Vaginal	525 (85.5)	375 (71.4)	0.58	151 (28.8)	0.46	102 (27.7)	0.77	124 (33.8)	0.99
Cesarean section	89 (14.5)	61 (68.5)		29 (32.6)		18 (29.5)		20 (33.9)	
Birth weight[b]									
< 3000 g	96 (15.6)	64 (66.7)	0.31	22 (22.9)	0.08	11 (19.6)	0.02	13 (23.6)	0.02
3000–4000 g	411 (66.7)	295 (71.8)		122 (29.7)		78 (26.4)		96 (32.8)	
> 4000 g	109 (17.7)	79 (72.5)		37 (33.9)		32 (40.0)		36 (45.0)	
Birth order (N = *614*)									
Has older siblings	359 (58.5)	276 (76.9)	< 0.001	97 (27.0)	0.14	76 (29.2)	0.47	91 (35.1)	0.47
First born	255 (41.5)	160 (62.7)		83 (32.5)		44 (26.0)		53 (31.7)	
Smokers living in the home at birth									
No	413 (67.1)	280 (67.8)	0.01	103 (24.9)	< 0.001	72 (24.5)	0.02	90 (30.8)	0.05
Yes	203 (33.0)	158 (77.8)		78 (38.4)		49 (35.8)		55 (40.4)	
Residence location									
Urban	336 (54.5)	229 (68.2)	0.08	88 (26.2)	0.06	62 (25.3)	0.14	77 (31.6)	0.24
Rural	280 (45.5)	209 (74.6)		93 (33.2)		59 (31.7)		68 (37.0)	
Maternal asthma									
No	516 (83.9)	353 (68.4)	0.001	144 (27.9)	0.06	97 (26.9)	0.21	116 (32.4)	0.12
Yes	99 (16.1)	84 (84.8)		37 (37.4)		24 (34.3)		29 (42.0)	
Breastfeeding (N = *613*)									
< 3 months exclusive	277 (45.2)	212 (76.5)	0.009	93 (33.6)	0.05	64 (35.6)	0.004	75 (42.1)	0.003
⩾ 3 months exclusive	336 (54.8)	225 (67.0)		88 (26.2)		57 (22.9)		70 (28.2)	
Infections in first year[c]									
Upper respiratory	504 (81.8)	412 (81.7)	< 0.001	151 (30.0)	0.55	106 (30.5)	0.20	127 (36.6)	0.18
Lower respiratory	215 (34.9)	190 (88.4)	< 0.001	67 (31.2)	0.46	42 (27.6)	0.38	49 (32.7)	0.42
Non-respiratory	278 (45.1)	213 (76.6)	< 0.001	92 (33.1)	0.30	59 (30.7)	0.20	68 (35.4)	0.25
Any	555 (90.1)	428 (77.1)	< 0.001	165 (29.7)	0.57	112 (28.9)	0.27	134 (34.7)	0.27
None	61 (9.9)	10 (16.4)		16 (26.2)		9 (20.9)		11 (26.2)	
Antibiotics in first year									
No	178 (28.9)			37 (20.8)	0.003	24 (18.2)	0.002	33 (25.2)	0.01
Yes	438 (71.1)			144 (32.9)		97 (32.4)		112 (37.7)	
Maternal overweight (N = *497*)									
No	210 (42.3)	137 (65.2)	0.02	40 (19.0)	< 0.001	32 (19.2)	0.001	40 (24.1)	0.001
Yes	287 (57.7)	214 (74.6)		97 (33.8)		81 (34.0)		95 (40.3)	
Reported at age 9:									
Asthma—age 9									
No	398 (64.6)	273 (68.6)	0.06	112 (28.1)	0.36	69 (25.8)	0.17	86 (32.5)	0.43
Yes	218 (35.4)	165 (75.7)		69 (31.7)		52 (31.9)		59 (36.2)	
Number of siblings[d]									
0	49 (8.0)	27 (55.1)	0.003	17 (34.7)	0.05	15 (46.9)	0.03	18 (56.3)	0.01
1	310 (50.3)	213 (68.7)		91 (29.4)		60 (26.6)		66 (29.5)	
⩾ 2	257 (41.7)	198 (77.0)		73 (28.4)		46 (26.6)		61 (35.5)	
Annual family income (N = *600*)[b]									
< $30 000	48 (8.0)	37 (77.1)	0.30	21 (43.8)	0.002	12 (41.4)	0.10	13 (44.8)	0.05
$30 000 to < $60 000	176 (29.3)	126 (71.6)		60 (34.1)		36 (29.8)		46 (38.3)	
⩾ $60 000	376 (62.7)	262 (69.7)		96 (25.5)		71 (26.3)		82 (30.1)	
Daily screen time (N = *614*)[b]									
< 1 h	144 (23.5)	95 (66.0)	0.22	29 (20.1)	0.001	13 (13.0)	0.001	22 (22.0)	0.001
1 to < 3 h	397 (64.7)	289 (72.8)		122 (30.7)		87 (31.6)		96 (35.3)	
3 or more hours	73 (11.9)	54 (74.0)		30 (41.1)		21 (38.2)		27 (49.1)	

Table. 2. (Continued)

	Overall prevalence N = *616*[a]	*Exposed to antibiotics during first year* N = *616*[a]		*Overweight at age 9* N = *616*[a]		*Overweight at age 12* N = *431*[a]		*High central adiposity at age 12* N = *428*[a]	
	n *(%)*	n *(%)*	P	n *(%)*	P	n *(%)*	P	n *(%)*	P
Vigorous physical activity (N = *614*)									
⩾3 days per week	496 (80.8)	355 (71.6)	0.79	129 (26.0)	< 0.001	94 (26.8)	0.19	117 (33.5)	0.69
0–2 days per week	118 (19.2)	83 (70.3)		52 (44.1)		27 (34.2)		28 (35.9)	
Milk consumption (N = *613*)									
0–2 days per week	52 (8.5)	34 (65.4)	0.33	23 (44.2)	0.02	16 (41.0)	0.06	19 (50.0)	0.03
⩾3 days per week	561 (91.5)	403 (71.8)		157 (28.0)		105 (26.9)		126 (32.5)	
Vegetable consumption (N = *613*)									
0–2 days per week	63 (10.3)	49 (77.8)	0.23	25 (39.7)	0.06	12 (27.9)	0.96	20 (46.5)	0.07
⩾3 days per week	550 (89.7)	388 (70.5)		155 (28.2)		109 (28.2)		125 (32.6)	
Fast food consumption (N = *612*)									
0–2 days per week	581 (94.9)	411 (70.7)	0.24	170 (29.3)	0.72	114 (27.9)	0.40	137 (33.7)	0.35
⩾3 days per week	31 (5.1)	25 (80.6)		10 (32.3)		7 (36.8)		8 (44.4)	
Reported at age 12:									
Asthma—age 12 (N = *431*)									
No	294 (68.2)	195 (66.3)	0.04	79 (26.9)	0.33	76 (25.9)	0.13	93 (31.9)	0.19
Yes	137 (31.8)	104 (75.9)		43 (31.4)		45 (32.9)		52 (38.2)	
YHEI Diet Quality Score (N = *423*)									
< Median	207 (48.9)	147 (71.0)	0.39	58 (28.0)	0.96	59 (28.6)	0.55	71 (34.5)	0.60
⩾ Median	216 (51.1)	145 (67.1)		60 (27.8)		56 (26.1)		68 (32.1)	

Abbreviation: YHEI, Youth Healthy Eating Index. [a]Unless otherwise indicated. [b]Comparison by Cochrane-Armitage test for trend (all other comparisons by chi-squared test). [c]No infections as reference group. [d]Total number of siblings (older and younger) at age 7.

Table 3. Risk of childhood overweight and central adiposity following antibiotic exposure in the first year of life

Outcome Model	*All children*		*Girls*		*Boys*	
	N	*OR (95% CI)*	N	*OR (95% CI)*	N	*OR (95% CI)*
Overweight—age 9						
Crude association	616	1.87 (1.23–2.82)	270	1.76 (0.96–3.23)	346	1.91 (1.08–3.38)
Adjusted model 1[a]	595	1.79 (1.15–2.79)	262	1.49 (0.77–2.87)	333	2.07 (1.10–3.88)
Adjusted model 2[b]	484	1.74 (1.04–2.94)	210	1.20 (0.53–2.70)	274	2.19 (1.06–4.54)
Overweight—age 12						
Crude association	431	2.16 (1.31–3.58)	182	1.35 (0.66–2.78)	249	3.16 (1.51–6.60)
Adjusted model 1[a]	408	2.24 (1.26–3.97)	174	1.21 (0.52–2.82)	234	4.04 (1.68–9.69)
Adjusted model 2[b]	386	2.56 (1.36–4.79)	164	1.13 (0.46–2.81)	222	5.35 (1.94–14.72)
High central adiposity—age 12						
Crude association	428	1.80 (1.14–2.85)	180	1.57 (0.79–3.11)	248	1.93 (1.03–3.61)
Adjusted model 1[a]	405	1.86 (1.11–3.13)	383	1.49 (0.68–3.25)	233	2.59 (1.21–5.54)
Adjusted model 2[b]	383	2.10 (1.19–3.70)	162	1.59 (0.68–3.68)	221	2.85 (1.24–6.51)

Abbreviations: OR, odds ratio; CI, confidence interval. [a]Adjusted for birth weight, breastfeeding, smoke exposure at birth, family income, sibship (any siblings at age 7), diet (milk consumption at age 9 or Youth Healthy Eating Index score at age 12), physical activity at age 9, current asthma, maternal asthma. [b]Adjusted for all covariates in Model 1, plus maternal overweight.

therefore, we also examined associations in subsamples stratified by gender and maternal overweight.

RESULTS

Prevalence of antibiotic exposure, overweight and central adiposity

Among the 616 children studied, over two-thirds (71.1%) received at least one antibiotic prescription before their first birthday (Table 1). Of these, 95 (15.4%) received more than four courses of antibiotics, and 73 (11.9%) received their first antibiotic prescription before 3 months of age. The majority of antibiotics prescribed were broad spectrum, with only 13 infants (2.1%) exposed to narrow-spectrum antibiotics alone.

The prevalence of childhood overweight was 29.4% at age 9 (181/616), and 28.1% at age 12 (121/431) (Table 1). The prevalence of high central adiposity at age 12 was 33.9% (145/428). Children who were lost to follow-up by 12 years of age ($n = 185$, 30.0%) were more likely to have a low birth weight and less likely to have been exclusively breastfed beyond 3 months, but were not significantly different from the continuing study population in

terms of antibiotic use, overweight, central adiposity or other demographic characteristics (Supplementary Table S1).

Crude association of antibiotic exposure, overweight and central adiposity

Infants receiving antibiotics were significantly more likely to become overweight later in childhood compared with those who were unexposed (Table 2). By age 9, 32.9% of children receiving antibiotics during infancy had become overweight, compared with 20.8% of unexposed children ($P = 0.003$). Similarly at age 12, the prevalence of overweight (32.4 versus 18.2%, $P = 0.002$) and high central adiposity (37.7 versus 25.2%, $P = 0.01$) were significantly higher among children receiving antibiotics in their first year of life (Table 1).

Adjustment for potential confounders

Infant antibiotic exposure was associated with several known obesity risk factors,[43] including maternal overweight, environmental tobacco smoke exposure and short duration of exclusive breastfeeding (Table 2). As expected, these factors were also

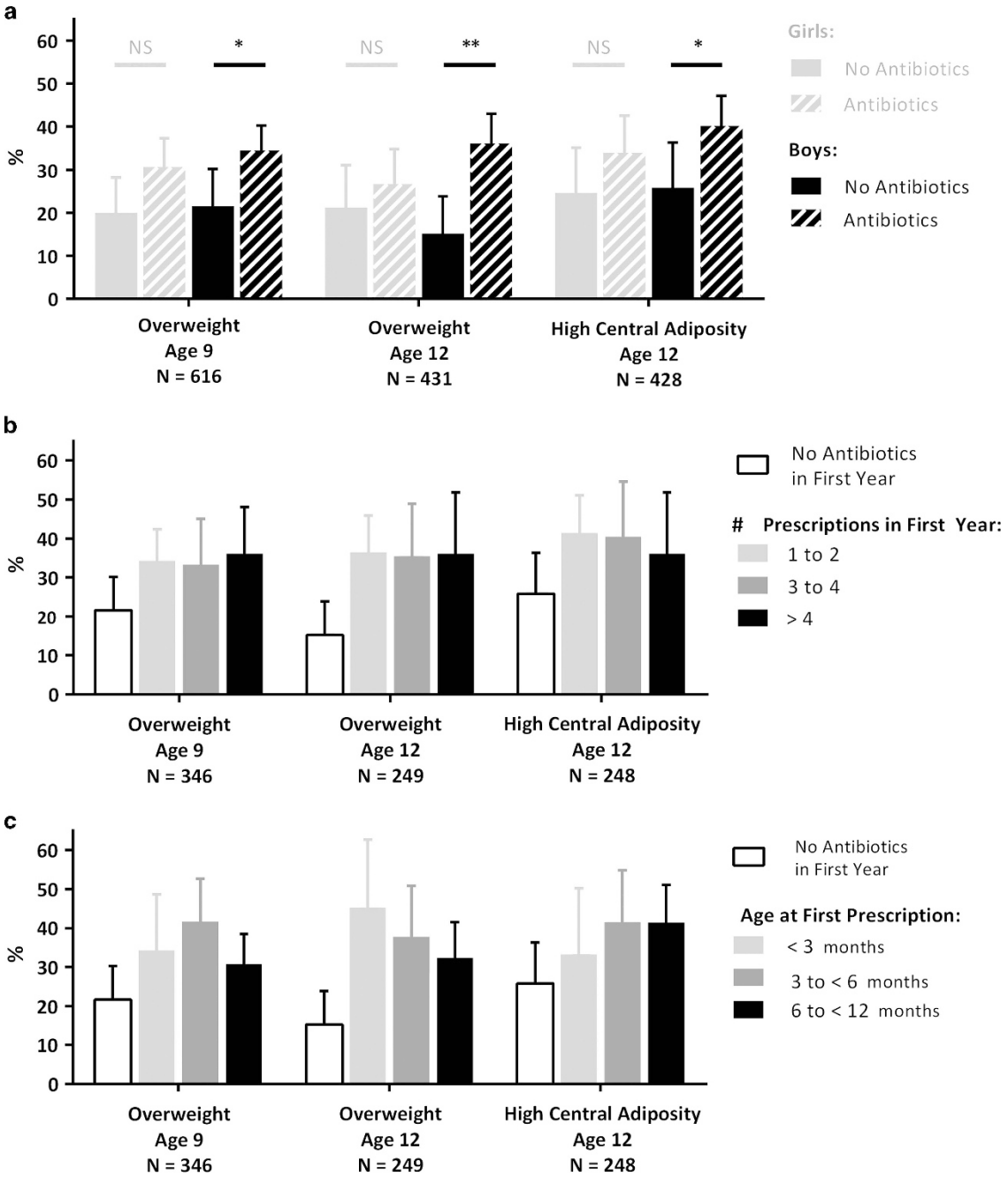

Figure 1. Unadjusted prevalence estimates of childhood overweight and high central adiposity according to early-life antibiotic exposures. (**a**) Any infant antibiotic exposure, stratified by gender; (**b**) frequency of antibiotic exposure, boys only; (**c**) timing of first antibiotic exposure, boys only. Antibiotic exposures during the first year of life were documented from provincial prescription records, and classified according to the number of courses received during this period, and the timing of first exposure. Direct measurement of child height and weight (ages 9 and 12) and waist circumference (age 12) were used to define overweight (>85th percentile BMI) and high central adiposity (>75th percentile waist circumference). Error bars indicate upper limits of 95% CIs. Comparisons by chi-squared test (A: NS, no significant difference; $^{*}P < 0.05$; $^{**}P < 0.01$) or Cochrane-Armitage test for trend (B and C: no significant trends among exposed children).

strongly associated with child overweight and adiposity (Table 2). After adjusting for these and other relevant covariates (birth weight, family income, sibship, child and maternal asthma, and measures of child diet and physical activity), we found that infant antibiotic exposure remained independently associated with childhood overweight and central adiposity (Table 3): adjusted OR (aOR) 1.74 (95% confidence interval (CI), 1.04–2.94) for overweight at age 9, aOR 2.56 (95% CI,1.36–4.79) for overweight at age 12, and aOR 2.10 (95% CI, 1.19–3.70) for high central adiposity at age 12. Results were unchanged following further adjustment for early-life infections (not shown).

Stratified analyses based on *a priori* hypotheses for interaction

Gender stratification revealed that associations of infant antibiotic exposure and childhood overweight were stronger among boys (Table 3, Figures 1 and 2). In boys, infant antibiotic exposure was associated with a five-fold increased risk of overweight at age 12 (aOR 5.35, CI 1.94–14.72), whereas there was no association in girls (aOR 1.13, CI 0.46–2.81) (*P* for interaction, 0.05). Similar gender-specific associations were found for overweight at age 9 and for high central adiposity at age 12 (Table 3, Figure 2). These patterns of association persisted following additional adjustment for pubertal status (not shown).

We did not find any evidence of interaction between infant antibiotic use and maternal overweight status ($P > 0.40$ for all outcomes). The association of infant antibiotic exposure and overweight at age 9 was comparable among children born to normal weight (aOR 1.96, CI 0.80–4.77) and overweight mothers (aOR 1.62, CI 0.85–3.12). Similarly, comparable associations were found for overweight and high central adiposity at age 12, with no evidence of interaction with maternal weight, even when analyses were restricted to boys only (not shown).

Frequency, timing and type of infant antibiotic exposure

Compared with unexposed infants, the risk of overweight by age 9 was similarly elevated when the first antibiotic exposure occurred before 6 months of age (aOR 1.63, CI 0.91–2.93) or between 6 and 12 months of age (aOR 1.85, CI 1.05–3.25). The 6-month cut point was evaluated for comparability with a previous study;[25] assessment of more precise exposure time windows (< 3 months, 3 to < 6 months and 6 to < 12 months) yielded the largest OR with the earliest exposure for overweight at age 12, but this pattern was not observed for central adiposity or for overweight at age 9 (Table 4 and Figure 1c). We did not find evidence of a dose–response relationship, as associations of infant antibiotic exposure and childhood overweight outcomes were similar regardless of the number of antibiotic courses received (Table 4 and Figure 1b). Finally, because of the small number of children receiving narrow-spectrum antibiotics, we lacked statistical power to detect differences in overweight status according to antibiotic spectrum; however, similar associations were observed for exposure to broad-spectrum antibiotics only (aOR 2.48, CI 1.31–4.68 for overweight at age 12), or narrow-spectrum antibiotics with or without broad-spectrum antibiotics (aOR 3.14, CI 1.30–7.62).

DISCUSSION

Using detailed prescription records linked with direct anthropometric measurements from a longitudinal birth cohort study, we found that exposure to antibiotics in the first year of life was associated with a significantly increased risk of overweight and central adiposity at ages 9 and 12, among boys only. Regardless of birth weight, maternal overweight and other potential confounders, male infants receiving antibiotics were more than five times as likely (aOR 5.35, CI 1.94–14.72) to become overweight by age 12, compared with males who were unexposed. They were also more than twice as likely to develop high central adiposity (aOR 2.85, CI 1.24–6.51), putting them at increased risk for metabolic and cardiovascular disease.[27,29,30,36] Notably, these associations were independent of established obesity risk factors including diet, physical activity and maternal overweight.

Although the precise mechanisms are poorly understood, it is widely accepted that growth promotion by antibiotics is mediated through interaction with the gut microbiota[44] because antibiotic-enhanced growth is not seen in microbiota-free animals.[45] Intestinal microbes and their metabolic products can affect weight gain and fat deposition through a variety of mechanisms, such as influencing gut hormones to modify appetite and satiety, regulating insulin action and glucose metabolism,[46] stimulating low-grade inflammation,[47] and increasing host energy absorption by fermenting otherwise indigestible foods.[6] Indeed, both human and animal studies have found that gut microbiota composition differs between obese and non-obese individuals.[8,48–51] Recent evidence from Luoto *et al.*[52] indicates that such differences may be established during early life, because microbiota composition at 3 months of age predicted overweight 10 years later. These studies, together with evidence that antibiotic exposure causes extensive and long-lasting perturbations to the intestinal microbiota,[10,11] have propagated the notion that antibiotic

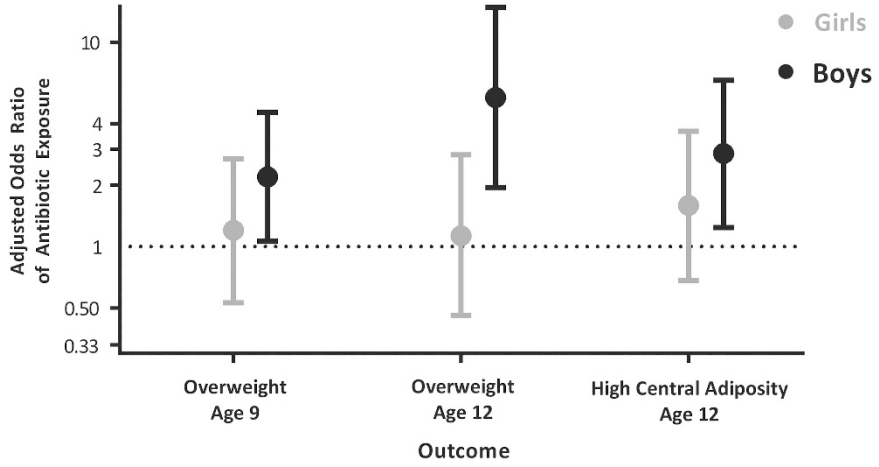

Figure 2. Adjusted likelihood of overweight and high central adiposity in girls and boys at ages 9 and 12 according to antibiotic exposure in the first year of life. Error bars represent 95% CIs, corresponding to aORs from fully adjusted models in Table 3 (adjusted for birth weight, smoke exposure, breastfeeding, family income, sibship, child and maternal asthma, maternal overweight, and child diet and physical activity).

Table 4. Risk of childhood overweight and adiposity following antibiotic exposure during the first year of life, according to number of antibiotic courses and timing of first exposure

Outcome Exposure	*All Children* *aOR (95%CI)*	*Girls* *aOR (95%CI)*	*Boys* *aOR (95%CI)*
Overweight—age 9	*N* = 484	*N* = 210	*N* = 274
Any antibiotics in first year	1.74 (1.04–2.94)	1.20 (0.53–2.70)	2.19 (1.06–4.54)
Number of courses[a]			
None (reference)	1.00	1.00	1.00
1–2	2.02 (1.15–3.55)	1.89 (0.76–4.75)	2.15 (0.99–4.65)
3–4	1.26 (0.64–2.48)	0.46 (0.14–1.51)	2.25 (0.91–5.57)
>4	1.77 (0.87–3.62)	1.42 (0.42–4.78)	2.26 (0.88–5.82)
	P trend = 0.47	*P* trend = 0.19	*P* trend = 0.77
Age at first antibiotic prescription			
No antibiotics (reference)	1.00	1.00	1.00
< 3 months	1.34 (0.63–2.83)	1.28 (0.40–4.13)	1.48 (0.53–4.14)
3 to < 6 months	1.83 (0.96–3.49)	0.60 (0.20–1.82)	3.70 (1.54–8.92)
6 to < 12 months	1.85 (1.05–3.26)	1.66 (0.67–4.10)	1.91 (0.87–4.21)
Overweight—age 12	*N* = 386	*N* = 164	*N* = 222
Any antibiotics in first year	2.56 (1.36–4.79)	1.13 (0.46–2.81)	5.35 (1.94–14.72)
Number of courses[a]			
None	1.00	1.00	1.00
1–2	3.07 (1.57–6.00)	1.44 (0.52–4.03)	5.86 (2.08–16.56)
3–4	2.12 (0.98–4.61)	1.04 (0.31–3.43)	4.60 (1.42–14.90)
>4	1.85 (0.78–4.37)	0.68 (0.15–2.99)	4.46 (1.30–15.36)
	P trend = 0.13	*P* trend = 0.39	*P* trend = 0.37
Age at first antibiotic prescription			
No antibiotics	1.00	1.00	1.00
< 3 months	3.25 (1.40–7.52)	2.01 (0.57–7.12)	6.73 (1.88–24.08)
3 to < 6 months	1.72 (0.79–3.76)	0.36 (0.09–1.47)	5.48 (1.72–17.51)
6 to < 12 months	2.85 (1.45–5.58)	1.48 (0.52–4.20)	5.06 (1.78–14.42)
High Central Adiposity—age 12	*N* = 383	*N* = 162	*N* = 221
Any antibiotics in first year	2.10 (1.19–3.70)	1.59 (0.68–3.68)	2.85 (1.24–6.51)
Number of courses[a]			
None	1.00	1.00	1.00
1–2	2.70 (1.47–4.95)	2.33 (0.91–5.94)	3.22 (1.36–7.64)
3–4	1.41 (0.68–2.92)	0.64 (0.19–2.13)	2.60 (0.95–7.13)
>4	1.64 (0.74–3.65)	1.86 (0.49–7.02)	1.98 (0.67–5.89)
	P trend = 0.07	*P* trend = 0.34	*P* trend = 0.29
Age at first antibiotic prescription			
No antibiotics	1.00	1.00	1.00
< 3 months	1.33 (0.58–3.05)	1.37 (0.40–4.69)	1.41 (0.42–4.71)
3 to < 6 months	1.68 (0.83–3.41)	1.15 (0.38–3.52)	2.58 (0.96–6.95)
6 to < 12 months	2.65 (1.44–4.87)	2.07 (0.79–5.41)	3.34 (1.41–7.93)

Abbreviations: aOR, adjusted odds ratio (adjusted for covariates corresponding to Model 2 in Table 3: birth weight, breastfeeding, smoke exposure at birth, family income, sibship, diet, physical activity, current asthma, maternal asthma and maternal overweight); CI, confidence interval. [a]Test for linear trend according to number of antibiotic courses was conducted among exposed children; associated *P*-values are shown.

exposure may influence the development of overweight. Our current findings contribute new evidence to support this emerging hypothesis.

Three previous human studies have investigated the association of early-life antibiotic use and childhood overweight. Using data from the Danish National Birth Cohort (DNBC), Ajslev *et al.*[24] found that early-life antibiotic use was associated with a 20% increased risk of overweight at age 7. Transande *et al.*[25] published findings from the UK Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, showing that antibiotic use in the first 6 months was associated with a 22% elevated risk of overweight and consistent increases in body mass up to 38 months of age. Most recently, Murphy *et al.*[26] analyzed cross-sectional data from the International Study of Asthma and Allergies in Childhood (ISAAC) and found that antibiotic exposure during the first 12 months was associated with increased BMI in boys aged 5–8 years. Our findings at 9 and 12 years of age further confirm this association and demonstrate, for the first time, long-lasting effects into pre-adolescence. Moreover, ours is the first study to demonstrate an association of early-life antibiotic exposure and central adiposity, which is considered a better predictor of obesity-related health outcomes than BMI-based total adiposity.[27–30]

Both the DNBC and ISAAC studies reported gender-specific associations, finding that infant antibiotic exposure was independently associated with subsequent measures of overweight among boys, but not girls.[24] Our current findings are consistent with these reports, showing strong associations among boys only. The biological explanation for this gender specificity remains to be determined, but could be related to sex-specific differences in antibiotic drug metabolism or microbiota–host interactions.[53] Commensal gut microbes contribute to the metabolism of sex steroid hormones,[54] which in turn regulate the accumulation and distribution of adipose tissues.[55] A recent study showed that sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity;[56] our current finding of sex-specific antibiotic effects on overweight could also be related to sex differences in the gut microbiome, but further research will be required to explore this potential mechanism. Our finding of a larger effect size for overweight (aOR 5.35) compared to central adiposity (aOR 2.85) may also provide mechanistic insights,

suggesting that antibiotic growth-promoting effects apply to both muscle and fat mass.

The ALSPAC and DNBC studies reported effect modification by maternal overweight, with greater effects of antibiotic exposure among infants born to normal-weight versus overweight mothers.[24,25] We did not detect such an interaction in our cohort, for either overweight or central adiposity, perhaps because of the differences in the prevalence or classification of maternal overweight. The prevalence of maternal pre-pregnancy overweight in ALSPAC and DNBC was 15% and 23%, respectively.[24,25] In our cohort, maternal pre-pregnancy overweight was estimated from data collected 10–12 years post partum and prevalence was 58%, consistent with provincial statistics.[57] Trasande *et al.*[25] reported that earlier antibiotic exposure (<6 months) had a stronger impact than later exposure (6–12 months) on the development of overweight, but this pattern of association was not observed in our cohort. Similar to Murphy *et al*,[26] we detected a consistent effect of antibiotic exposure occurring any time during the first 12 months.

Our results demonstrate that infant antibiotic exposure is a risk factor for child overweight and central adiposity, independent of many established early-life determinants of obesity. As others have shown,[43] we found that exclusive breastfeeding beyond 3 months was protective against child overweight and central adiposity. Measures of birth weight, maternal overweight, parental smoking, sibship, childhood diet and physical activity were also associated with overweight, as expected. Antibiotic effects were independent of these exposures, suggesting that traditionally protective factors (breastfeeding, healthy diet and physical activity) do not reverse the obesogenic effects of antibiotic exposure during infancy, and that classic risk factors (maternal overweight, high birth weight, parental smoking) do not explain the association of infant antibiotic exposure and child overweight in boys. Although our cohort overrepresented mothers and children with asthma, we adjusted for and found no significant association between maternal or child asthma and overweight, indicating that our findings are broadly applicable to the general population. Finally, our findings were unchanged following adjustment for early-life infections, but confounding by (antibiotic) indication is difficult to control for in analyses. On the other hand, the strong association with sibship, a proxy measure of exposure to microbes or viruses, did not diminish the antibiotic 'effect' on childhood overweight and central adiposity.

The major strengths of this study are the complete, accurate and detailed characterization of antibiotic use from provincial prescription records, the direct measurement of child height, weight and waist circumference, and the extended follow-up to 12 years of age (previous studies relied on parent report of medication use, and none measured waist circumference or collected outcome data beyond 8 years of age).[24–26] We were also able to adjust for multiple potential confounding factors, derived from health-care records and survey data (e.g., birth weight, infections, breastfeeding, diet, physical activity, maternal weight and environmental tobacco smoke exposure).

Our study was limited by a relatively smaller sample size compared with previous studies, and by attrition, with 30% loss to follow-up by 12 years of age; however, antibiotic exposure and initial measures of overweight were comparable among participants with and without complete follow-up data. We did not have information on maternal pre-pregnancy BMI, an important predictor of overweight in offspring[58] reflecting a combination of genetic, *in utero* and lifestyle-related mechanisms; however, we did capture and adjust for maternal BMI at 12 years postpartum, recognizing that it is an imperfect but reasonable proxy measure for pre-pregnancy BMI. Also, although antibiotic prescriptions were fully captured and likely represent the majority of exposures, we were unable to document antibiotics administered in hospital, as these are not captured in the provincial prescription database. In a recent review of hospital records for Manitoba infants,[59] we found that fewer than 6% of infants received antibiotics in hospital during their first year of life; therefore, underestimation of antibiotic exposure in the current study would be minimal.

In summary, our results provide new evidence that infant antibiotic exposure is associated with an increased risk of total and central adiposity in pre-adolescence, among boys only. These findings imply that the first year of life constitutes a critical window of development, where antibiotics may program metabolic physiology and later obesity, potentially through mechanisms involving the gut microbiota. Given the current epidemic of childhood obesity, and the high prevalence of infant antibiotic exposure, further research is needed to determine the mechanisms underlying this association, to identify the long-term implications for metabolic and cardiovascular health, and to develop strategies for mitigating these effects when antibiotic exposure cannot be avoided. In the meantime, our results provide evidence for long-term consequences of early antibiotic exposure, and underscore the need for stewardship programs to ensure appropriate prescribing of antibiotics during infancy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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