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Association of class number, cumulative exposure, and earlier initiation of antibiotics during the first two-years of life with subsequent childhood obesity

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ABSTRACT

Background: Despite the possible association between antibiotic overuse and childhood obesity, studies on this association are lacking in Asia. This study aimed to determine whether there is an association between the number, duration of antibiotic exposure, timing of antibiotics initiation and childhood obesity.
Methods: In this retrospective cohort study, Korean children born between January 1, 2008 and December 31, 2012, who underwent government-provided health examinations at age 4–6 and 30–36 months, were included. The main outcome was obesity (body mass index in 95th percentile) at 30–36 months. The exposure variable was antibiotic prescription during the first 24 months of life. The number, prevalence, and odds ratio (OR) of obese children based on antibiotic exposure were analyzed using logistic regression.
Results: Of 31,733 children, 31,457 (99.1%) children used antibiotics and 2843 (9%) were obese at 30–36 months. There was a clear dose-response relationship between obesity and number of antibiotic classes, cumulative days, and earlier antibiotic initiation. Children who used five or more antibiotic classes had higher odds of obesity than those who used only one class (OR 1.42, 95% CI 1.12–1.8). Children with >180 days of antibiotic exposure had higher risk of obesity than those with 1–30 exposure days (OR 1.40, 95% CI 1.19–1.64). Children with earlier initiation of antibiotics had higher risk of obesity (OR 1.15 per 6 months, 95% CI 1.08–1.22).
Conclusion: Increased number of antibiotic classes, longer duration of antibiotic prescription and earlier antibiotic initiation before 24 months was associated with childhood obesity at 30–36 months. This South Korean retrospective study supports judicious use of antibiotics in the first 24 months of life to avoid the potential risk of childhood obesity. Future studies need to be performed to confirm or refute the results presented herein.

1. Introduction

The prevalence of childhood obesity is continuously increasing worldwide, which has become a serious health problem [1–3]. Obesity has been considered a worldwide epidemic. It has already been proven that childhood obesity can lead to childhood hypertension, diabetes, dyslipidemia, and adult metabolic syndrome. Studies have suggested that childhood obesity can lead to adult obesity, which has serious implications on society [4–6]. About one-third (26%–41%) of obese preschool children and about one-half (42%–63%) of obese school-aged children remain obese as adults [7]. Although the imbalance between caloric uptake and expenditure, inappropriate diet quality, and lack of exercise are the main causes of childhood obesity, many other precipitating factors have been revealed [8].

Blaser et al. [9] asserted that antibiotics are a 4-edged sword. The first and second edges are that antibiotics become cure for individual infection as well as prevent the spread in the community. The third is antibiotics resistance and the fourth edge is alteration of gut microbiota, which causes unexpected health outcomes. The gut microbiota is composed of many microorganisms that exist symbiotically [10,11]. However, this active homeostatic community of microorganisms can easily be perturbed by medication use [12,13]. According to Cox et al. [14], in vivo experiments have indicated that microbiota-altering medications can influence the energy metabolism of animals by increasing cell adiposity. These medications include almost every class of

Abbreviations: OR, odds ratio; CI, confidence interval; SES, socioeconomic status; NHSIC, National Health Screening Program for Infants and Children; NHIS, National Health Insurance Service; ATC, Anatomical Therapeutic Chemical; BMI, body mass index; H2RAs, histamine-2-receptor antagonists; PPIs, proton pump inhibitors.

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antibiotics. Therefore, not only have early-stage antibiotics been used for enhancing the growth of cattle and other ruminants, but it has also been proven that antibiotics can increase the rate of weight gain in children with severe acute malnutrition [15].

Although there have been some human studies on antibiotics and weight gain [16–18], the results have been conflicting until recently. In a secondary analysis of data from a large clinical trial of trimethoprim-sulfamethoxazole prophylaxis, Edmonson et al. [16] found no evidence that prolonged exposure to this antibiotic significantly affects weight gain. Moreover, Gerber et al. [17] concluded that exposure to antibiotics within the first 6 months of life was not associated with a statistically significant difference in weight gain through age 7 years. Conversely, Trasande et al. [18] reported that antibiotic exposure during the first 6 months of life is associated with consistent increases in lean body mass from 10 to 38 months. Further, in a retrospective cohort study including infants born into the US Military Health System, Stark et al. [19] found that antibiotic prescription was associated with childhood obesity. These conflicting results have raised curiosity among researchers. In 2018, Miller et al. [20] performed a meta-analysis combining the results of studies on antibiotic prescription within 2 years of age and childhood obesity. This meta-analysis assessed 12 sets of results about the earliest age of exposure to any antibiotic and overweight or obesity at the latest age of outcome and found a marginal pooled odds ratio (OR) of 1.05 (95% confidence interval [CI] 1.00–1.11).

However, there are currently no studies about the association between antibiotic class prescription and childhood obesity among Asian children. Furthermore, few existing studies adjusted for key confounders such as breastfeeding status, SES, and comorbid indications for antibiotic use. Epidemiological and large cohort studies have provided increasing evidence on the overuse and misuse of antibiotics in Korea [21,22]. Given the extensive overuse of antibiotics, high prevalence of childhood obesity in Korea, and controversial results of previous studies, further studies investigating the impact of early antibiotic prescription on childhood obesity are warranted. This study aimed to retrospectively review infant cohort data from the National Health Screening Program for Infants and Children (NHSIC) of the National Health Insurance Service (NHIS) of Korea and to demonstrate the presence of a dose-response relationship among antibiotic class, cumulative days of antibiotic prescription, and childhood obesity.

2. Material and methods

2.1. Data sources and dataset description

We conducted a population-based cohort study of Korean children born from January 1, 2008, to December 31, 2012. This study was approved as an exempted study by the Seoul National University Hospital Institutional Review Board (approval number: E-1904-003-1021).

This was a birth cohort study based on the NHSIC database of the NHIS. The Korean government launched the NHSIC in November 2007 as a population surveillance system, with the goal of improving the health and well-being of children. Children undergo the NHSIC at ages 4–6 months (first), 9–12 months (second), 18–24 months (third), 30–36 months (fourth), 42–48 months (fifth), 54–60 months (sixth), and 66–71 months (seventh). The NHSIC is an optional examination, and children can participate up to a maximum of seven times from age 4 to 71 months. In this examination, physicians conduct anthropometric measurements as well as history taking, physical examination, screening for visual acuity, and health education [23,24].

2.2. Inclusion and exclusion criteria

The NHSIC provides a 5% random sample of the population of children born between 2008 and 2012 who underwent the first or second examination (n = 83,910). Among these, 51,712 infants underwent the first and fourth examinations (Fig. 1). Only infants who underwent the first examination had available birth weight information. Those who were born premature (before 37 weeks' gestation), were Low Birth Weight Infants (birth weight < 2500 g), and were hospitalized in the neonatal intensive care unit for ≥5 days were excluded from the analysis, to eliminate confounding data related to premature infant growth patterns and hospital course exposures due to perinatal complications that may undermine generalization [19]. Further, infants or children who had missing variables or uncertain birth date were excluded. Although the birth year was provided, the exact birth date was not revealed for privacy protection. Thus, minimum and maximum possible dates were estimated using the dates of health examinations, earliest date of insurance claim, and year of birth. Those whose estimated range of birth dates was >31 days were excluded. Finally, 31,733 infants were included in this study.

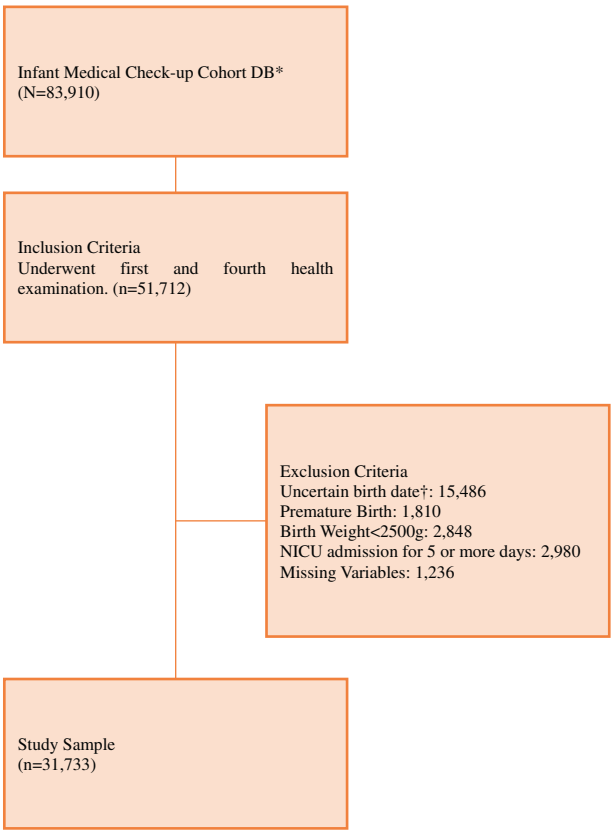

Fig. 1. Study sample selection. *Out of total check-up recipients who received at least one of 1st -2nd infant medical check-up, 5% of sample is extracted for each birth year of 2008–2012. †Birth date was not provided as part of the database for privacy and a minimum and maximum possible date was estimated using date of health examination, date of insurance claim, and year of birth. Those with estimated range of birth dates greater than 31 days were excluded.

2.3. Ascertainment of variables

2.3.1. Main outcome variable

Childhood obesity, the primary outcome for this study, was determined at the fourth examination (30–36 months). Obesity was defined based on the age- and sex-specific 95th percentile body mass index (BMI) z-scores of the general Korean population, which was released in 2017 by the Korea Centers for Disease Control and Prevention [25].

2.3.2. Ascertainment of antibiotic exposure

The exposure variables were the number of antibiotic class, cumulative prescription days, and age at first prescription in the first 24 months of life. Antibiotic exposure was determined using the claim

database for drug prescriptions. Macrolides, penicillin, cephalosporin, fluoroquinolones, sulfonamides, and others, including vancomycin and lincosamides, were defined according to the World Health Organization Anatomical Therapeutic Chemical (ATC) classification of drugs [26–28]. Number of classes was categorized to 0, 1, 2, 3, 4, or 5 or more classes. Cumulative days were categorized to 0, 1–30, 30–60, 60–90, 90–180, and 180 or more days. Age at first antibiotics was categorized into non-users, 18–24, 12–18, 6–12, and 0–6 months. Upon initial inspection of antibiotic use, the number of antibiotic non-users was insufficient to serve as an adequate reference for statistical analysis. Thus, the least-exposed categories, namely one antibiotic class, 1–30 days of exposure, and age 18–24 months at first prescription, were used as references. For additional analysis, we determined the effect of each antibiotic class by comparing its respective users versus non-users, whereby we determined the single class effect without adjustment for users of other classes. This was performed to determine if the use of any one class causes a significant risk increase. A supplementary analysis of number of class effect on obesity among subgroups of age at first prescription was performed to assess the temporal effect.

2.3.3. Ascertainment of covariates

Breastfeeding status was determined using the NHIS surveillance questionnaire given to mothers at 4–6 months. It was classified into four groups: only breastmilk feeding, only formula feeding, both breastmilk and formula feeding, and special formula. The parents' SES was defined by quartiles of insurance premium. Birth weight and pre-term birth status were determined using a survey at the first health examination. As antibiotic prescription patterns and obesity rates may differ by regions, we adjusted for place of residence, which was categorized into capital, metropolitan, and rural. Acid suppressant use, which may affect gut microbiota, were determined by either proton pump inhibitor prescription or histamine-2-receptor antagonist prescription as defined by the ATC classification of drugs. Possible comorbidities that may be associated with antibiotic prescription were determined. Histories of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, skin infection, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis were identified during the first 2 years of life using the International Classification of Diseases 10th edition diagnostic codes.

2.4. Statistical analysis

Multivariate logistic regression was used to calculate ORs with 95% CIs. Dose-response analysis was performed using categories showing the OR with 95% CI per unit increase in category. Statistical models were used separately for each main exposure, namely antibiotics class number, antibiotics cumulative prescription days, and age at first prescription of antibiotics. These exposures were not used simultaneously in the same model to prevent potential collinearity. Each main exposure was first analyzed using a univariate logistic model to determine the singular effect. Then a multivariate logistic model was used adjusting for concurrent drug use and baseline characteristics (Models 1, 3, and 5 in Table 2; Model 1 in Table 3; and Model 1 in Table 4). Finally, a multivariate logistic model was used adjusting for concurrent drug use, baseline characteristics, and antibiotics-related comorbidities (Models 2, 4, and 6 in Table 2; Model 2 in Table 3; and Model 2 in Table 4). Concurrent drug use and baseline characteristics include H2RA, PPI, sex, breastfeeding status, socioeconomic status, residence, and birth weight. Antibiotics related comorbidities include history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, skin infection, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis. A test for collinearity was performed for all models using variance inflation factors (VIF) and tolerance values [29]. Interaction tests between the primary exposure variables and covariates were performed. Statistical analyses were performed using SAS 9.4 (SAS Institute, Cary, NC, USA). A P-value of <0.05 was considered to indicate statistical significance.

3. Results

A total of 31,733 children met the study inclusion criteria. Each child was followed up until the BMI measurement at the fourth examination (30–36 months). The mean age at BMI measurement was 2.8 years. The median birth weight was 3.2 kg. The average number of antibiotic classes was 3.28 (standard deviation 1.08), and the average number of days of antibiotic prescription was 101.3 days (standard deviation 80.6 days). The number of antibiotic non-users was 276 (0.9%) (Table 1). Table 1 shows the characteristics of the study sample according to antibiotics class number and antibiotics cumulative days by sex, breastfeeding status, SES, birth weight, and residence. Appendix Table 1 shows the characteristics of the study sample by age at first antibiotic prescription.

The number of obese children and obesity prevalence among 10,000 individuals were 2843 and 896, respectively. The unadjusted OR and multivariable-adjusted OR of obesity were determined for antibiotics class number, antibiotics cumulative prescription days, and age at first prescription of antibiotics, in separate models (Table 2). There was a clear dose-response relationship between obesity with antibiotic class number (OR 1.09, 95% CI 1.05–1.14), antibiotic cumulative days (OR 1.06, 95% CI 1.03–1.08), and age at first prescription (OR 1.11, 95% CI 1.05–1.17). Children who used five or more antibiotic classes had higher odds of obesity than those who used only one class (OR 1.42, 95% CI 1.12–1.80). Children with >181 days of antibiotic exposure had a higher risk of obesity than those with 1–30 days of exposure (OR 1.40, 95% CI 1.19–1.64), with statistically significant increases in obesity risk from ≥61 days of exposure. Children with earlier initiation of antibiotics had higher risk of obesity (OR 1.15 per 6 months, 95% CI 1.08–1.22). No apparent collinearities were found between covariates used in logistic models (Appendix Table 2).

The unadjusted OR and multivariable-adjusted OR of obesity were determined for covariates are shown in Table 3. Although H2RAs and PPIs (acid suppressants) had increased ORs, these were not statistically significant in terms of 95% CIs. These results are further elaborated in Appendix Table 3, which shows that the acid-suppressant users did not have a significant increase in obesity compared with non-users even when stratified in subgroups according to days of antibiotic prescription, and Appendix Table 4, which shows the insignificant effect of acid suppressants on obesity. Obesity was more prevalent in male children, but was not statistically significant, and these effects were more attenuated when variables were adjusted in models 1 and 2. Formula feeding had a higher association with obesity than breastmilk feeding (OR 1.14, 95% CI 1.04–1.25) (Table 3). The OR increased more when both breastfeeding and formula feeding were provided. Although in previous studies, causality between formula feeding and obesity was obscured because SES was a confounding factor, in our study, there were significant ORs even in adjusted models. In addition to the infant's feeding status, the SES of the infant's parents also influenced childhood obesity. The odds of the lowest SES quartile for obesity were higher than those of the highest SES quartile (OR 1.20, 95% CI 1.06–1.36). The unadjusted odds of obesity was significantly higher among infants living in rural areas than in those living in the capital (OR, 1.14, 95% CI 1.02–1.28), but was attenuated after adjustments for antibiotic class number, H2RA use, PPI use, sex, breastfeeding status, and SES. Initial birth weight was significantly associated with obesity.

The proportion of obese children stratified by individual antibiotic classes was also investigated (Table 4). Macrolides (broad-spectrum antibiotics) had the largest OR among the antibiotic classes (OR 1.39, 95%

Table 1
Cohort characteristics.

	Total population	Antibiotics non-user	Antibiotics User				
			Number of antibiotics class		Number of cumulative days prescribed		
			1–3	≥ 4	1–60	61–120	≥ 121
Number (%)	31,733(100)	276(0.9)	14,444(45.5)	17,013(53.6)	11,568(36.5)	9739(30.7)	10,150(32)
Birth weight, kg, median (P25-P75)	3.2(3.0–3.5)	3.20(3.00–3.40)	3.2(3.0–3.5)	3.2(3.0–3.5)	3.2(3.0–3.5)	3.2(3.0–3.5)	3.2(3.0–3.5)
Age at measured BMI, years, median (P25-P75)	2.85 (2.65–3.00)	2.90 (2.67–3.02)	2.86 (2.65–3.00)	2.84 (2.64–3.00)	2.87 (2.66–3.01)	2.84 (2.65–3.00)	2.83 (2.64–2.99)
Male, N (%)	16,374 (51.6)	115 (41.7)	7038 (48.7)	9221 (54.2)	5526 (47.8)	5066 (52)	5667 (55.8)
Breastfeeding status							
Only breast milk	13,913 (43.8)	146 (52.9)	6494 (45)	7273 (42.7)	5290 (45.7)	4270 (43.8)	4207 (41.4)
Only formula milk	11,167 (35.2)	66 (23.9)	4845 (33.5)	6256 (36.8)	3742 (32.3)	3393 (34.8)	3966 (39.1)
Both	6494 (20.5)	63 (22.8)	3048 (21.1)	3383 (19.9)	2498 (21.6)	2018 (20.7)	1915 (18.9)
Special formula milk	159 (0.5)	1 (0.4)	57 (0.4)	101 (0.6)	38 (0.3)	58 (0.6)	62 (0.6)
Socioeconomic status							
Lowest Quartile	6277 (19.8)	40 (14.5)	2756 (19.1)	3481 (20.5)	2110 (18.2)	1971 (20.2)	2156 (21.2)
2nd Quartile	7472 (23.5)	57 (20.7)	3316 (23)	4099 (24.1)	2655 (23)	2289 (23.5)	2471 (24.3)
3rd Quartile	11,386 (35.9)	99 (35.9)	5187 (35.9)	6100 (35.9)	4131 (35.7)	3512 (36.1)	3644 (35.9)
Highest Quartile	6598 (20.8)	80 (29)	3185 (22.1)	3333 (19.6)	2672 (23.1)	1967 (20.2)	1879 (18.5)
Residence							
Capital	5919 (18.7)	84 (30.4)	3064 (21.2)	2771 (16.3)	2598 (22.5)	1757 (18)	1480 (14.6)
Metropolitan	17,082 (53.8)	149 (54)	7944 (55)	8989 (52.8)	6131 (53)	5220 (53.6)	5582 (55)
Rural	8732 (27.5)	43 (15.6)	3436 (23.8)	5253 (30.9)	2839 (24.5)	2762 (28.4)	3088 (30.4)

CI 1.26–1.53). After macrolides, the antibiotics that showed statistical significance were penicillin (OR 1.29, 95% CI 1.04–1.60), cephalosporin (OR 1.27, 95% CI 1.12–1.44), and fluoroquinolones (OR 1.22 95% CI 1.13–1.32), whereas sulfonamides, lincosamides, and vancomycin did not show statistically significant results. For adjusted ORs in model 2, macrolides (OR 1.26, 95% 1.14–1.40) and fluoroquinolones (OR 1.14, 95% CI 1.05–1.24), the broad-spectrum antibiotics, showed statistically significant increases in OR, suggesting that these antibiotic classes significantly increase the probability of childhood obesity.

Stratified analysis among lower and upper halves by initial birth weight showed a positive association between antibiotic exposure and obesity (Appendix Table 5). Furthermore, a supplementary analysis showing the obesity risk of antibiotics class number grouped by the age at first prescription (Appendix Table 6) showed similar increasing trends of obesity risk with increasing antibiotics class in all ages of initiation.

4. Discussion

This study showed a clear association between cumulative antibiotic prescription days, antibiotic class number, and earlier antibiotic initiation with childhood obesity from a large population-based cohort.

Our results agree with those of previous studies that showed a link between early-onset antibiotics and childhood obesity. Azad et al. [30] conducted a case-control study in 616 children who were exposed to antibiotics in the first year of life and underwent anthropometric measurements at ages 9 and 12. The association persisted only in male children. However, previous studies to date did not show consistent results regarding the association between sex and obesity [16–19]. Moreover, a secondary analysis of a clinical trial [16] found that long-term antibiotic prophylaxis (trimethoprim-sulfamethoxazole) for 2 years had no effect on weight gain in children with vesicoureteral reflux. However, the limitations of this study were the investigation of only one type of antibiotic and a relatively short follow-up (24 months). A retrospective longitudinal study [17] found that antibiotic prescription within the first 6 months of life had no statistically significant relationship to weight gain. In Bailey et al.'s study however, broad-spectrum antibiotic prescription within the first 24 months of life was statistically correlated with weight gain, rendering the use of broad-spectrum antibiotics a modifiable risk factor [31].

Our study showed a dose-response relationship between antibiotics usage before 24 months and subsequent childhood obesity at 30–36 months. Nevertheless, our study also showed that antibiotic prescription is almost ubiquitous in Korea reaching almost 99%. Those who did not use antibiotics at all were a mere 0.9% of the total population (276 of 31,733). Compared to other Organization for Economic Cooperation and Development (OECD) countries, antibiotic consumption in South Korea, especially in Korean children, is exceptionally high as Choe and Park et al.'s [21,22] studies pointed out. Because of antibiotic overuse, antibiotic resistance is also a problem, making antibiotic stewardship crucial [32,33]. Therefore, in our logistic regression analysis, we did not set antibiotic non-users as reference. Instead, one antibiotic class and 1–30 cumulative prescription days were set as references. Nonetheless, this did not change the fact that there was a clear dose-relationship between early-onset antibiotic usage and childhood obesity. The exact reason for this phenomenon is unknown, and the rationale for antibiotics treatment might depend on the individual physician rather than enforced guidelines. Therefore, it is difficult to know how carefully these antibiotics are being prescribed. Presumably, in Korea, where patients perceive medication prescription as a benefit, physicians may prescribe antibiotics in patients with mild upper respiratory tract symptoms for rapport although this is not recommended in clinical practice. It is difficult to present the exact mechanism to determine the appropriate use of antibiotics or a mechanism to reduce the use of antibiotics solely with administrative data. Restrictive policies such as reimbursement for the patient could be helpful but effective communication between the doctor and patients would be more efficient. As evidence regarding antimicrobial stewardship accumulates, effective communication and education of potential risks of antibiotics to the patients and caregiver will be the optimal choice.

Several mechanisms for the association between antibiotic prescription and childhood obesity have been proposed. Interestingly, the intestinal microbiota has been suggested as a key contributor to disease risks and could partly explain the link between antibiotics and childhood obesity [19,34]. Ley et al. [35] revealed that the proportion of *Bacteroidetes* in the gut microbiome decreases in obese patients, and that higher ratio of *Firmicutes*/*Bacteroidetes* were found in intestinal microbiota of obese patients, when compared to lean, normal weight individuals. Antibiotics could affect the gut microbiota [12,13] and altered gut microbiota could affect obesity [14].

Because of the pervasiveness that antibiotic itself has on gut microbiota, the use of antibiotics in early life and excessive weight gain in later childhood could possibly be linked. This has extensively been investigated in animal studies as many of Korpela et al.'s studies [36–38]

Table 2
Risk of obesity by antibiotics class number, cumulative days, and age at first prescription.

	Number of obese (%)	Prevalence per 10 K	Unadjusted OR (95% CI)	Model 1 adjusted OR (95% CI)	Model 2 adjusted OR (95% CI)
All	2843(9.0)	896			
Antibiotics class number[a]			1.13 (1.09–1.18)	1.12 (1.08–1.16)	1.09 (1.05–1.14)
0	15(5.4)	543	0.82 (0.48–1.43)	0.86 (0.5–1.50)	0.81 (0.46–1.43)
1	126(6.5)	652	ref	ref	ref
2	433(7.6)	755	1.17 (0.95–1.44)	1.14 (0.93–1.40)	1.12 (0.91–1.38)
3	614(9.1)	906	1.43 (1.17–1.74)	1.37 (1.13–1.68)	1.3 (1.06–1.6)
4	1422(9.6)	964	1.53 (1.27–1.85)	1.46 (1.21–1.76)	1.34 (1.1–1.64)
5 or more	233(10.3)	1029	1.64 (1.31–2.06)	1.58 (1.26–1.98)	1.42 (1.12–1.8)

	Number of obese (%)	Prevalence per 10 K	Unadjusted OR (95% CI)	Model 3 adjusted OR (95% CI)	Model 4 adjusted OR (95% CI)
Antibiotics cumulative days[b]			1.08 (1.06–1.10)	1.07 (1.05–1.09)	1.06 (1.03–1.08)
0	15(5.4)	543	0.74 (0.43–1.25)	0.78 (0.46–1.33)	0.72 (0.42–1.24)
1–30	379(7.2)	720	ref	ref	ref
31–60	499(7.9)	792	1.11 (0.97–1.27)	1.09 (0.95–1.25)	1.08 (0.93–1.24)
61–90	489(8.9)	894	1.27 (1.10–1.46)	1.23 (1.07–1.42)	1.20 (1.03–1.39)
91–120	387(9.1)	906	1.28 (1.11–1.49)	1.23 (1.06–1.43)	1.19 (1.01–1.40)
121–150	319(10.1)	1015	1.46 (1.25–1.70)	1.40 (1.19–1.64)	1.33 (1.12–1.58)
151–180	240(10.6)	1064	1.54 (1.30–1.82)	1.46 (1.23–1.73)	1.38 (1.15–1.67)
>180	515(10.8)	1084	1.57 (1.36–1.80)	1.49 (1.29–1.71)	1.40 (1.19–1.64)

	Number of obese (%)	Prevalence per 10 K	Unadjusted OR (95% CI)	Model 5 adjusted OR (95% CI)	Model 6 adjusted OR (95% CI)
Age at first prescription of antibiotics[c]			1.22 (1.15–1.30)	1.19 (1.12–1.26)	1.15 (1.08–1.22)
Non-user	15(5.4)	543	0.85 (0.46–1.56)	0.85 (0.46–1.58)	0.82 (0.44–1.53)
18–24 months	39(6.4)	635	ref	ref	ref
12–18 months	217(7.6)	760	1.21 (0.85–1.73)	1.16 (0.81–1.66)	1.13 (0.79–1.62)
06–12 months	978(8.3)	826	1.33 (0.95–1.85)	1.24 (0.89–1.73)	1.17 (0.83–1.63)
00–06 months	1594(9.9)	987	1.62 (1.16–2.24)	1.47 (1.05–2.04)	1.33 (0.95–1.86)

Abbreviations; H2RA, histamine 2 receptor antagonist; PPI, proton pump inhibitor; OR, odds ratio; CI, confidence interval.
Model 1 Adjusted for antibiotics group number, H2RA, PPI, sex, breastfeeding status, socioeconomic status, residence, and birth weight.
Model 2 Adjusted for Model 1 plus history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, infection of the skin, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis.
Model 3 Adjusted for antibiotics cumulative days, H2RA, PPI, sex, breastfeeding status, socioeconomic status, residence, and birth weight.
Model 4 Adjusted for Model 3 plus history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, infection of the skin, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis.
Model 5 Adjusted for age at first prescription of antibiotics, H2RA, PPI, sex, breastfeeding status, socioeconomic status, residence, and birth weight.
Model 6 Adjusted for Model 5 plus history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, infection of the skin, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis.
[a] OR for trend represents OR per unit increase in number of antibiotics classes prescribed. ORs for antibiotics class number does not adjust for antibiotics cumulative days nor age at first prescription to prevent collinearity.
[b] OR for trend represents OR per unit increase in categories which are divided into 0, 1–30, 31–60, 61–90, 91–120, 121–150, 151–180, and 181 or more days. ORs for antibiotics cumulative days does not adjust for antibiotics class number nor age at first prescription to prevent collinearity.
[c] OR for trend represents OR per 6 months earlier initiation of antibiotics. ORs for age at first prescription of antibiotics does not adjust for antibiotics class number nor antibiotics cumulative days to prevent collinearity.

endeavor to prove. Antibiotic prescription during infancy, when the microbiota is still being established and is easily affected by other stimulations, could be detrimental to a child's metabolism and biological characteristics [39]. In a previous study, ciprofloxacin decreased the gut microbiota diversity and shifted the community composition immensely and rapidly within 3–4 days of drug initiation [40]. Therefore, the influence and change of the gut microbiota could explain the mechanism of the effect of antibiotics on childhood obesity.

The ascertainment of covariates such as initial birth weight, breastfeeding status, SES, and residence are distinct characteristics of this study. Initial birth weight, which could be a potential confounding factor, was adjusted and the association between antibiotics usage and obesity persisted in the adjusted models (Table 2). Breastfeeding was associated with lower risk of obesity compared to formula feeding in our study, as several other studies have shown [41,42]. This may be explained by alteration of intestinal microbiota [38]. Children of parents with higher SES had a lower probability of becoming obese, which is an already well-established fact. Moreover, although obesity augmented with increasing rurality in unadjusted models, this effect was attenuated after adjusting for other confounding variables such as antibiotic class number, acid-suppressant use, and SES. These findings correlate with those of previous studies in the United States and China [43–45], which suggested that living in a rural environment could increase the risk of obesity. However, these differences could also be influenced by differences in prescription methods or SES. Overall, our results on initial birth weight, breastfeeding status, SES, and residence largely agree with those of previous studies; thus, supporting the validity of our sample.

Stark et al.'s study [19] revealed that acid suppressants along with antibiotics has the potential to change the gut microbiota. Taking this study into consideration, our study also adjusted acid suppressant use along with antibiotics. However, our study reflected statistically insignificant changes in the odds ratios of obesity when acid suppressants were used (Appendix Tables 3, 4).

In terms of individual antibiotic classes, the phenomenon of broad-spectrum antibiotics such as macrolides and fluoroquinolones having larger ORs compared with other antibiotic classes strengthens the study conducted by Bailey et al. [31] and recommends physicians to prescribe broad-spectrum antibiotics only when absolutely necessary. The antibiotics for each antibiotic classes are presented in Appendix Table 7. Although the risk of sulfonamides, lincosamides, and vancomycin did not

Table 3
Obesity risk by concurrent drug use, birth weight, sex, breastfeeding status, socioeconomic status, and residence.

	Number of obese (%)	Prevalence per 10 K	Unadjusted OR (95% CI)	Model 1 adjusted OR (95% CI)	Model 2 adjusted OR (95% CI)
All	2843(9.0)	896			
H2RA	256(9.4)	937	1.07 (0.95–1.22)	1.03 (0.91–1.17)	1.02 (0.9–1.16)
PPI	22(11.7)	1170	1.32 (0.96–1.82)	1.32 (0.95–1.83)	1.31 (0.94–1.82)
Birth weight[a]			1.40 (1.35–1.45)	1.41 (1.36–1.46)	1.40 (1.35–1.46)
Q1 (<3.0 kg)	351(5.2)	524	ref	ref	ref
Q2 (3.0–3.2 kg)	723(7.2)	723	1.41 (1.24–1.61)	1.42 (1.24–1.62)	1.41 (1.24–1.61)
Q3 (3.2–3.5 kg)	568(9.5)	951	1.9 (1.66–2.19)	1.92 (1.67–2.21)	1.92 (1.67–2.2)
Q4 (≥3.5 kg)	1201(13.3)	1326	2.77 (2.45–3.13)	2.79 (2.46–3.15)	2.77 (2.45–3.14)
Sex					
Male	1508(9.2)	921	1.07 (0.99–1.15)	0.95 (0.88–1.03)	0.94 (0.87–1.02)
Female	1335(8.7)	869	ref	ref	ref
Breastfeeding status					
Only breast	1152(8.3)	828	ref	ref	ref
Only formula	1029(9.2)	921	1.12 (1.03–1.23)	1.14 (1.04–1.25)	1.14 (1.04–1.25)
Both	647(10.0)	996	1.23 (1.11–1.36)	1.24 (1.12–1.38)	1.24 (1.12–1.38)
Special formula	15(9.4)	943	1.15 (0.68–1.97)	1.10 (0.64–1.89)	1.09 (0.64–1.88)
Socioeconomic status					
Lowest Quartile	609(9.7)	970	1.24 (1.09–1.40)	1.23 (1.08–1.39)	1.21 (1.07–1.37)
2nd Quartile	714(9.6)	956	1.22 (1.08–1.37)	1.20 (1.07–1.35)	1.20 (1.06–1.35)
3rd Quartile	992(8.7)	871	1.10 (0.98–1.23)	1.10 (0.98–1.23)	1.09 (0.98–1.22)
Highest Quartile	528(8.0)	800	ref	ref	ref
Residence					
Capital	494(8.3)	835	ref	ref	ref
Metropolitan	1527(8.9)	894	1.08 (0.97–1.20)	1.04 (0.94–1.16)	1.04 (0.93–1.16)
Rural	822(9.4)	941	1.14 (1.02–1.28)	1.07 (0.95–1.21)	1.07 (0.95–1.21)

Abbreviations; H2RA, histamine 2 receptor antagonist; PPI, proton pump inhibitor; OR, odds ratio; CI, confidence interval.
Model 1 Adjusted for antibiotics group number, H2RA, PPI, sex, breastfeeding status, socioeconomic status, residence, and birth weight.
Model 2 Adjusted for Model 1 plus history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, infection of the skin, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis.
[a] OR for trend represents OR per unit increase in quartile category of birth weight.

significantly increase the risk of obesity, we cannot rule out a type II error due to the small number of users of these drugs (Table 4).

This study implemented the novel use of Korean NHIS data, which is a population-based representative sample in Korea. The fact that the number of antibiotic classes, number of antibiotic usage days, and antibiotic initiation age have been analyzed is a strength of this study. Increasing antibiotic number of classes, cumulative days, and earlier initiation all increased the risk of subsequent obesity. This particular phenomenon related to the timing of antibiotics may be explained by change in gut microbiota – the earlier the influence of antibiotics on gut microbiota, the larger the effect on subsequent obesity. Interestingly, regardless of antibiotics initiation timing, number of antibiotic classes increased the risk of obesity. The possibility of selection bias exists because we selected children who had

Table 4
Obesity risk by antibiotics class exposure compared to non-user of antibiotics.

Exposure variable	Number	Prevalence, /10000	Unadjusted OR (95%CI)	Model 1 adjusted OR (95%CI)	Model 2 adjusted OR (95%CI)
Penicillin (including amoxicillin and ampicillin)					
Non-user	1287	715	Ref	Ref	Ref
User	30,446	904	**1.29 (1.04–1.6)**	1.24 (1.00–1.54)	1.14 (0.91–1.43)
Cephalosporin					
Non-user	4017	739	Ref	Ref	Ref
User	27,716	919	**1.27 (1.12–1.44)**	**1.22 (1.07–1.38)**	1.13 (0.99–1.29)
Macrolide					
Non-user	7389	705	Ref	Ref	Ref
User	24,344	954	**1.39 (1.26–1.53)**	**1.35 (1.22–1.50)**	**1.26 (1.14–1.40)**
Fluoroquinolones					
Non-user	13,763	804	Ref	Ref	Ref
User	17,970	966	**1.22 (1.13–1.32)**	**1.20 (1.11–1.30)**	**1.14 (1.05–1.24)**
Sulfonamides					
Non-user	28,191	891	Ref	Ref	Ref
User	3542	937	1.06 (0.94–1.19)	1.06 (0.94–1.20)	1.02 (0.90–1.15)
Others[a]					
Non-user	31,693	896	Ref	Ref	Ref
User	40	750	0.82 (0.25–2.67)	0.74 (0.23–2.43)	0.71 (0.22–2.33)

Abbreviations; OR, odds ratio; CI, confidence interval.
Model 1 Adjusted for respective exposure variable, acid suppressant use, sex, breastfeeding status, socioeconomic status, residence, and birth weight.
Model 2 Adjusted for Model 1 plus history of acute upper respiratory infection, burns, chronic sinusitis, influenza and pneumonia, intestinal infection, suppurative otitis media, allergic rhinitis, viral infections with skin lesions, asthma, bronchitis, otitis externa, infection of the skin, chronic rhinitis or nasopharyngitis, urethritis, cystitis, viral infection of the central nervous system, and acute tubulointerstitial nephritis.
Non-users represent the non-users of the respective antibiotic class and not the non-user of all antibiotics.
Bold text means statistically significant odds ratios, where the 95% confidence intervals do not include 1.
[a] Others includes lincosamides and vancomycin.

undergone the first and fourth NHSIC health examination, which may affect results. However, the health examinations are provided free of charge at the national level and thus provide little bias regarding household income or medical accessibility. Furthermore, compared to other studies of this work which were single center studies [16,30] or of military families [19], the random sample selection from a national cohort of all Korean children is a strength of our study. Finally, the fact that our study relied on a large population sample is also a strength of this study.

This study had some limitations. Firstly, although we adjusted for potential confounders and biases, because this study was an observational retrospective cohort study, this study does not necessarily indicate causality but rather shows dose-response associations between antibiotics usage and childhood obesity. Secondly, information about the exact gut microbiota composition, which could be influenced by antibiotics, was not investigated. Thirdly, the follow-up duration of this study (30–36 months) was relatively short compared to other studies. Moreover, because the accumulated administrative data is from prescriptions, and we could not ascertain the use of prescribed medication. Also, data on the mode of birth delivery, maternal weight, smoking status, caloric intake, and other comorbid health problems were not available for this retrospective cohort study. In particular, maternal health status was not available because of the anonymization of the NHIS database and because the NHIS database does not provide information regarding the child's whole family. This is an inherent limitation of retrospective administrative data and warrants further work with prospective design. Regarding mode of birth delivery, previous studies suggested that babies born via cesarean section could have different microbiota compositions compared to vaginal delivery, and that both cesarean section and prenatal antibiotics are independent risk factors for childhood obesity which could affect our results [46,47]. As with any retrospective study, there is a chance for type 1 error, or the possibility of false positive results. However, our work agrees with previous studies addressing antibiotics use and obesity association [19,31]. Furthermore, our results were consistent in multiple models which addressed potential confounders and the risk of obesity showed a dose-response relationship with three different types of proxy variables for antibiotics.

5. Conclusion

Among Korean newborns, antibiotic prescription before 24 months of life was associated with subsequent childhood obesity with a significant dose-dependent relationship. Increasing number of antibiotics class, increasing duration of antibiotic prescription and earlier initiation of antibiotics was associated with an increased risk of obesity. This South Korean retrospective study supports judicious use of antibiotics in the first 24 months of life to avoid the potential risk of childhood obesity. The possibility of uncontrolled confounding by factors not studied herein still remains and future work is needed to confirm or refute the results presented herein.

Contributors

Dr. SM Park has full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Declaration of competing interest

None.

Appendix A. Supplementary data

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