Early-Life Antibiotic Exposure, Gut Microbiota Development, and Predisposition to Obesity

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Abstract

Antibiotics are often prescribed inappropriately to infants and young children, with potentially adverse effects on the developing gut microbiota and related metabolic processes. We review evidence from 17 epidemiologic studies suggesting that antibiotic exposure during critical periods of early development may influence weight gain and the development of obesity. Complementary research in both humans and rodents indicates that gut microbiota play a key role in this process, although further research is needed to confirm and characterize the causal mechanisms involved. Obesity is a complex and multifactorial condition; thus, a multipronged prevention strategy will be required to curb the current obesity epidemic. Evidence to date suggests this strategy should include the judicious use of antibiotics, especially in early life when the developing gut microbiota is particularly susceptible to perturbations with long-lasting implications for metabolic programming and obesity risk.

Introduction

Obesity is a major public health challenge in both developed and developing countries. Global obesity prevalence is expected to reach 20% by 2025 \cite{1}, placing over 1 billion individuals at risk for obesity-related complications, including cardiovascular disease and diabetes. Accumulating evidence indicates that
weight gain trajectories are “programmed” in early life, and that gut microbiota play a key role in this process [2–5]. Antibiotics could therefore impact weight gain and obesity risk by disrupting the normal colonization and development of gut microbiota during critical phases of prenatal and postnatal development. Here, we summarize the evidence for this hypothesis from recent epidemiologic and clinical studies, as well as experimental research in rodent models (Fig. 1).

Epidemiologic Evidence: Early-Life Antibiotics and Subsequent Obesity

Antibiotics have been used as growth promoters in the farming industry for decades [6], but this phenomenon had not been studied in humans until 2011 when Ajslev et al. [7] reported an increased risk of overweight among school-age children who had received antibiotics during infancy. Several subsequent studies in different settings have provided further evidence of this association (Table 1).

Three population-based prospective cohort studies in the UK [8], Denmark [7], and The Netherlands [9] have found that antibiotic exposure during the first 6 months of life is significantly associated with increased weight gain,
Table 1. Summary of epidemiologic studies evaluating early-life antibiotic exposure and anthropometric outcomes

<table>
<thead>
<tr>
<th>Study, year</th>
<th>Setting</th>
<th>n</th>
<th>Timing of antibiotic exposure</th>
<th>Anthropometric outcome</th>
<th>Age at outcome assessment</th>
<th>Main finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajslev et al. [7], 2011</td>
<td>Denmark</td>
<td>28,354</td>
<td>&lt;6 months</td>
<td>Overweight, obesity</td>
<td>7 years</td>
<td>Increased risk of overweight, only in children of normal-weight mothers</td>
</tr>
<tr>
<td>Trasande et al. [8], 2013</td>
<td>UK</td>
<td>11,532</td>
<td>&lt;24 months</td>
<td>BMI, overweight, obesity</td>
<td>0–7 years</td>
<td>Increased BMI and risk of overweight with exposure before 6 months</td>
</tr>
<tr>
<td>Bailey et al. [12], 2014</td>
<td>USA</td>
<td>64,580</td>
<td>&lt;24 months</td>
<td>BMI, obesity</td>
<td>2–5 years</td>
<td>Increased risk of obesity; cumulative effects; stronger effects with earlier exposures</td>
</tr>
<tr>
<td>Azad et al. [17], 2014</td>
<td>Canada</td>
<td>616</td>
<td>&lt;12 months</td>
<td>Overweight, central adiposity</td>
<td>12 years</td>
<td>Increased risk of overweight and high central adiposity, only in boys</td>
</tr>
<tr>
<td>Murphy et al. [18], 2014</td>
<td>18 countries</td>
<td>74,946</td>
<td>&lt;12 months</td>
<td>BMI</td>
<td>5–8 years</td>
<td>Increased BMI, only in boys</td>
</tr>
<tr>
<td>Mor et al. [19], 2015</td>
<td>Denmark</td>
<td>9,886</td>
<td>In utero</td>
<td>Overweight, obesity</td>
<td>7–16 years</td>
<td>Increased risk of overweight and obesity; stronger effects in boys; differences according to birth weight</td>
</tr>
<tr>
<td>Mueller et al. [20], 2015</td>
<td>USA</td>
<td>436</td>
<td>In utero</td>
<td>BMI, obesity, adiposity</td>
<td>7 years</td>
<td>Increased BMI, adiposity, and risk of obesity</td>
</tr>
<tr>
<td>Krenz-Niedbala et al. [21], 2015</td>
<td>Poland</td>
<td>1,277</td>
<td>&lt;12 months</td>
<td>Obesity</td>
<td>8 years</td>
<td>Increased risk of obesity</td>
</tr>
<tr>
<td>Saari et al. [13], 2015</td>
<td>Finland</td>
<td>12,062</td>
<td>&lt;24 months</td>
<td>BMI, height, weight, obesity</td>
<td>0–2 years</td>
<td>Increased BMI; stronger effects in boys, with earlier exposures, and for macrolides</td>
</tr>
<tr>
<td>Gerber et al. [11], 2016</td>
<td>USA</td>
<td>38,614</td>
<td>&lt;6 months</td>
<td>Weight gain</td>
<td>0–8 years</td>
<td>No association</td>
</tr>
<tr>
<td>Mbakwa et al. [9], 2016</td>
<td>The Netherlands</td>
<td>979</td>
<td>&lt;10 years</td>
<td>Height, weight, BMI, overweight</td>
<td>0–10 years</td>
<td>Increased weight and height with exposure before 24 months; no association with BMI or overweight</td>
</tr>
<tr>
<td>Scott et al. [14], 2016</td>
<td>UK</td>
<td>21,714</td>
<td>&lt;24 months</td>
<td>Obesity</td>
<td>4 years</td>
<td>Increased risk of obesity; cumulative effects</td>
</tr>
<tr>
<td>Schwartz et al. [10], 2016</td>
<td>USA</td>
<td>163,820</td>
<td>3–18 years</td>
<td>BMI trajectories</td>
<td>3–18 years</td>
<td>Increased weight gain; reversible, persistent and progressive effects</td>
</tr>
<tr>
<td>Li et al. [16], 2017</td>
<td>USA</td>
<td>260,556</td>
<td>&lt;12 months</td>
<td>Obesity</td>
<td>1–18 years</td>
<td>Infections, not antibiotics, associated with obesity</td>
</tr>
</tbody>
</table>
overweight, or obesity later in childhood. Exposure later in infancy (after 6 months) was not consistently associated with weight gain in these studies [8, 9]. However, a recent longitudinal analysis of over 160,000 US children [10] demonstrated cumulative and persistent effects of antibiotic use on body mass index (BMI) trajectories from 3 to 15 years of age, suggesting that antibiotic use beyond infancy may continue to influence weight gain throughout childhood.

In contrast to the studies above, a registry-based US cohort study by Gerber et al. [11] found that antibiotic exposures during infancy (first 6 months) were not significantly associated with weight gain trajectories through 8 years of age. However, other registry-based studies [10, 12–15] have shown results supporting modest effects of early-life antibiotic exposure on weight gain and obesity risk. Some of these studies have also demonstrated dose-response gradients, with stronger associations from multiple exposures [12, 14, 15] and broad-spectrum antibiotics [12–15]. Most recently, findings from a large registry-based cohort study suggest that early-life infections, rather than antibiotics, are associated with an increased risk of subsequent obesity [16]. Given the limitations of administrative data sources used for registry-based studies, the observed associations are likely biased by inadequate control for confounders (such as breastfeeding, diet, and physical activity) and potential misclassification of weight-related outcomes or antibiotic exposures. However, these limitations likely bias the associations towards the null, as evidenced by the larger effect sizes reported in studies where some of these limitations were addressed [17, 18]. Notably, most of these epidemiologic studies did not distinguish between lean mass and fat mass, which is an important limitation since animal studies suggest that an-

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</tr>
</thead>
<tbody>
<tr>
<td>Edmonson et al. [22] 2017</td>
<td>USA</td>
<td>428</td>
<td>2 months to 7 years</td>
<td>Weight, overweight, obesity</td>
<td>2–7 years</td>
<td>No association. (population 92% female)</td>
</tr>
<tr>
<td>Poulsen et al. [15] 2017</td>
<td>USA</td>
<td>8,793</td>
<td>In utero and &lt;36 months</td>
<td>BMI</td>
<td>3 years</td>
<td>Increased BMI with postnatal exposure; cumulative effects; strongest with macrolides; no association with prenatal exposure</td>
</tr>
<tr>
<td>Ville et al. [23] 2017</td>
<td>USA</td>
<td>97</td>
<td>&lt;6 months</td>
<td>Obesity</td>
<td>2 years</td>
<td>Increased risk of obesity</td>
</tr>
</tbody>
</table>

BMI, body mass index. Studies are listed in order of publication date.
tibiotics may induce increased adiposity even without a change in overall body weight (see Experimental Evidence: Antibiotics, Microbiota, and Obesity in Animal Models).

New evidence suggests that in utero exposure to antibiotics may also influence weight gain. In a study of nearly 10,000 Danish school children, Mor et al. [19] reported an increased prevalence of obesity among those with prenatal exposure to antibiotics. Consistent with these findings, Mueller et al. [20] found that antibiotic exposure during gestation was associated with an increased risk of obesity at 7 years of age, along with higher BMI, waist circumference, and percent body fat. However, Poulsen et al. [15] found no association between prenatal antibiotic exposure and infant BMI at 3 years of age.

There is some evidence that antibiotic effects may be modified by infant sex or maternal BMI. Several studies have reported stronger associations in males [13, 17–19], although others found no sex differences [9, 15]. One study found that antibiotics were associated with increased obesity risk among children of normal weight mothers, while a protective association was observed among children of overweight mothers [7]. The mechanisms underlying these apparent interactions remain unexplained but may involve gut microbiota since both sex and maternal obesity are known to influence the establishment of the infant microbiota [5].

Adding to the growing body of observational evidence, Edmonson and Eickhoff [22] recently analyzed data from a randomized, placebo-controlled trial of prolonged antibiotic prophylaxis among young children at risk for recurrent urinary tract infections. In this secondary analysis, 24 months of daily trimethoprim-sulfamethoxazole treatment had no effect on weight gain or obesity. However, a single (nonmacrolide) antibiotic was tested, the median age at enrolment was 12 months, and 92% of participants were female. These factors might explain the lack of association, since observational studies have shown stronger associations from macrolides, early exposure (before 6 months), and among male infants (Table 1).

Despite the epidemiologic evidence that early-life antibiotic exposure may be associated with increased weight gain, adiposity, and obesity risk, the effect sizes are modest and the underlying mechanisms remain poorly understood. However, the hypothesized role of microbiota is supported by evidence linking gut microbiota with metabolic dysfunction, weight gain, and obesity (see Infant Gut Microbiota and Obesity), and by studies demonstrating that early antibiotic exposure can significantly and permanently alter gut microbiota profiles (see Antibiotics and the Developing Gut Microbiota). Experiments in animal models provide further supporting evidence and afford opportunities to study causal mechanisms (see Experimental Evidence: Antibiotics, Microbiota, and Obesity in Animal Models).
Colonization of the gastrointestinal tract is critical for neonatal development and has a lasting impact on long-term health [3, 24]. This process likely begins in utero with prenatal inoculation of the infant microbiota via transmission of bacteria through the placenta and amniotic fluid [25]. Further transmission occurs through exposure to vaginal and gut microbiota during birth and milk microbiota during breastfeeding [3, 26, 27]. Initially, the neonatal microbiota is dominated by *Bifidobacteria* before gradually developing through a series of successions and replacements into a more complex and adult-like microbiota by 2 years of age [28].

Once established, the human gut microbiota can be viewed as a metabolic organ that contributes to host weight gain through several mechanisms. Gut microbiota ferment indigestible complex carbohydrates into short-chain fatty acids (including propionate, butyrate, and acetate) that can be readily used by the host colonocytes as an energy source [5]. Short-chain fatty acids and other microbial metabolites can also influence the secretion of gut-derived peptides, which consequently regulate gut motility, nutrient absorption, satiety, and energy homeostasis [29]. Finally, disturbance of gut microbiota can affect the integrity and function of the gut, resulting in translocation of lipopolysaccharides to the bloodstream and triggering low-grade inflammation, a condition that characterizes obesity and other metabolic disorders [29].

Firmicutes and Bacteroidetes are the two most abundant phyla in the mature gut microbiota. Multiple studies have reported a relative increase in Firmicutes and a decrease in Bacteroidetes among obese individuals [24, 30, 31]. Further, Ley et al. [31] demonstrated that the relative abundance of Firmicutes decreased and Bacteroidetes increased following weight loss in human subjects. At lower taxonomic levels, obesity has been associated with higher abundance of the Enterobacteriaceae family, *Prevotella*, *Clostridium*, *Eubacterium*, and *Roseburia* genera, and *Faecalibacterium prausnitzii*, and lower abundance of the genus *Bifidobacterium* [5, 30]. While the majority of this evidence has arisen from cross-sectional studies that cannot determine whether alterations in microbiota composition are a cause or consequence of weight gain, an increasing number of longitudinal studies are demonstrating that alterations in gut microbiota precede the development of obesity. Intriguingly, these changes can sometimes be detected as early as the first weeks or months of life among infants who gain excessive weight later in childhood [4, 32].

Emerging evidence suggests that disruption of normal gut microbiota development early in life may foster an “obesogenic” microbiota that contributes to the subsequent development of obesity [26]. Summarizing evidence from 8
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studies investigating the association between maternal and infant gut microbiota and childhood obesity, Kozyrskyj et al. [5] concluded that higher proportions of *Lactobacillus* and lower proportions of *Bacteroides* within 3 months of birth predict a higher risk of becoming overweight later in childhood. In addition, a high abundance of *Bifidobacterium* spp. in early life appears to be associated with a lower risk of becoming overweight, whereas high abundance of *Bacteroides fragilis* increases the likelihood of obesity development [32]. Moreover, one of the strongest predictors of childhood obesity is maternal obesity [33], and a body of evidence suggests this may be partially explained by the vertical transfer of obesogenic microbiota from obese mothers to their offspring [5, 33].

With mounting evidence that gut microbiota contributes to weight gain and obesity, there is increasing interest in targeting or manipulating gut microbiota to prevent obesity or facilitate weight loss. Recognizing the importance of early gut colonization, several studies are testing new probiotic therapies (e.g. *Akkermansia muciniphila* and *Butyricicoccus pullicaeorum*) in clinical trials of pregnant women [4], although further investigations are needed to fully determine the clinical safety and effectiveness of this approach. Alongside these attempts to optimize gut microbiota with probiotics, there is a strong interest in understanding and mitigating the impact of antibiotics on the developing gut microbiota (see next section).

**Antibiotics and the Developing Gut Microbiota**

Gut colonization is influenced by perinatal and postnatal antibiotic exposure [26, 32]. Even the relatively stable and resilient adult gut microbiota can undergo persistent changes following repeated antibiotic exposures [34], but the infant gut microbiota is particularly susceptible given its transient developing state.

In many settings, intrapartum antibiotics are routinely administered as prophylaxis to women who are carriers of group B streptococci and to women delivering by cesarean section. Several small studies have demonstrated that antibiotic treatment during the intrapartum and early postnatal period can influence the developing gut microbiota. Fouhy et al. [35] reported that neonates treated with intravenous antibiotics (ampicillin and gentamicin) had higher proportions of *Proteobacterium* spp. and lower proportions of *Actinobacterium* and *Lactobacillus* spp. than untreated controls. These differences were detectable 4 weeks after treatment, and while most changes had resolved by 8 weeks, the shift in Proteobacteria persisted. Consistent with these results, Arboleya et al. [36] found that both maternal intrapartum antibiotics and neonatal antibi-
otic treatment were associated with an increased abundance of Enterobacteriaceae during the first 3 months of life. Maternal intrapartum antibiotics have also been associated with depletion of Bifidobacterium spp. and reduced bacterial richness 1 week after birth [37]. Recent results from the larger Canadian Healthy Infant Longitudinal Development (CHILD) study further demonstrate the impact of intrapartum antibiotics on infant microbiota, showing depletion of Bacteroides and enrichment of Enterococcus at 3 months of age [38].

Studies in older infants and children also show long-lasting effects of early antibiotic exposure. Bokulich et al. [28] recently profiled the gut microbiota development of 43 US infants during the first 2 years of life and found that antibiotic exposure during the first 12 months was associated with delayed microbiota maturation, characterized by the depletion of Lachnospiraceae and Erysipelotrichaceae, which in turn altered the functional capacity of the microbial community. Korpela et al. [39] examined antibiotic-induced alterations of gut microbiota in 142 Finnish children (2–7 years old) and found that macrolide use was associated with long-lasting shifts in gut microbiota, including depletion of Actinobacteria and enrichment of Bacteroidetes and Proteobacteria. Overall microbiota richness and maturity were also reduced and remained lower than in controls, even 2 years after exposure. Weaker effects and more rapid recovery were observed in the Finnish study following penicillin versus macrolide exposure, indicating that different antibiotics have distinct effects on gut microbiota. In both studies, stronger effects were observed when exposure occurred in the first 6 months of life, suggesting that a critical window exists where antibiotic exposure is particularly damaging to the developing gut microbiota.

Surprisingly little is known about the impact of in utero antibiotic exposure on the infant gut microbiota, although this is an area of growing interest given the widespread use of antibiotics during pregnancy [40], increasing knowledge of maternal-infant transfer of microbiota (see Infant Gut Microbiota and Obesity), and emerging evidence that even prenatal antibiotic exposure is associated with the risk of obesity (see Epidemiologic Evidence: Early-Life Antibiotics and Subsequent Obesity).

Experimental Evidence: Antibiotics, Microbiota, and Obesity in Animal Models

Animal models provide the opportunity to precisely control the dose and timing of antibiotic exposure, determine their impact on weight gain, and study the underlying biological mechanisms, including the role of gut microbiota. Blaser and his group have characterized the impact of early-life antibiotic exposure in a se-
eries of experiments using rodent models [41–44]. Initially, mice were given sub-therapeutic antibiotic treatment (STAT) with penicillin, vancomycin, or chlor-tetracycline to mimic the regular practice in farm animals [41]. STAT was initiated at weaning (4 weeks of age) and did not affect total body mass; however, a significant increase in fat mass and percent body fat was observed with penicillin or chlor-tetracycline treatment. Fecal microbiota composition was also substan-
tially modified by STAT, including an increase in the ratio of Firmicutes to Bac-
teroidetes [41].

Next, to examine the importance of the timing of antibiotic exposure, STAT was initiated either at birth or at weaning [42]. Accelerated growth, increased total body mass, and elevated abdominal and visceral adiposity were observed when mice were exposed from birth, while lesser effects were seen with later exposure. Weight gain and fat mass accumulation were further enhanced in STAT mice when a high-fat diet was introduced in adulthood. Consistent with epidemiologic findings (see Epidemiologic Evidence: Early-Life Antibiotics and Sub-
sequent Obesity), more pronounced effects were seen in males for some outcome measures. STAT also perturbed the fecal microbiota, including relative enrichment of Bacteroidetes and Proteobacteria and depletion of Firmicutes. At the genus level, Anaeroplasma, Coprobacillus, Oscillospira, and Ruminococcus were enriched in STAT mice while Lactobacillus, Prevotella, Allobaculum, and Candidatus Arthromatus were enriched in controls. Interestingly, STAT-in-
duced microbiota shifts generally resolved after STAT exposure was terminated, yet the metabolic phenotypes persisted. These sustained effects on body composition indicate that microbiota disruption in early life can have a long-term impact on the obesity risk, even when the microbiota appears to recover from the perturbation.

Microbiota transplant experiments were performed to demonstrate a causal role for microbiota in the obesogenic effect of early-life STAT [42], showing that germ-free mice colonized with microbiota from STAT mice gained more weight and fat mass than controls, despite never being directly exposed to antibiotics. Mechanisms for these obesogenic effects were explored in subsequent experiments, where bomb calorimetry showed that STAT did not affect eating behavior or energy harvest in mice on a high-fat diet [43]; however, STAT mice had increased insulin resistance and altered metabolic and inflammatory profiles. In addition, STAT was associated with distinct shifts in microbiota during wean-
ing, including a bloom of Proteobacteria, not seen in controls. STAT also sig-
ificantly delayed the maturation of microbiota during the first 4 weeks of life [43].

Although prolonged STAT is routine practice on animal farms, it does not accurately reflect human usage of antibiotics. To mimic patterns of pediatric
antibiotic use, a mouse model of pulsed antibiotic treatment at full therapeutic dose was established using amoxicillin (a β-lactam) or tylosin (a macrolide), reflecting the 2 most commonly prescribed antibiotic classes in children [44]. Both antibiotics moderately increased lean mass compared to controls, and, in the long term, tylosin induced a 60% greater weight change, although fat mass was not significantly affected. While the first pulse of antibiotics did not affect the microbiota maturation, the second and third pulses significantly delayed maturation compared to controls. Both antibiotics reduced the richness and evenness of bacterial communities in the short term. While amoxicillin-treated mice recovered their microbiota diversity soon after the last dose and developed a mature microbiota similar to controls, an immature and less diverse microbiota persisted in tylosin-treated mice. Similar results were recently reported by Kaliannan et al. [45], where pulsed early-life treatment with azithromycin (another macrolide) followed by a Western diet challenge led to increased weight gain, adiposity, insulin resistance, and a higher Firmicutes/Bacteroidetes ratio compared to untreated controls.

Studies examining perinatal antibiotic exposure in rodent models have also shown disruption of gut microbiota. Tormo-Badia et al. [46] treated pregnant mice with a cocktail of metronidazole, neomycin, and polymyxin and observed a persistent reduction in gut microbiota diversity among offspring. Gonzalez-Perez et al. [47] administered ampicillin, streptomycin, and clindamycin during gestation and lactation, and observed a reduction in total bacterial load accompanied by enrichment of Enterococcus species in the gut microbiota of offspring. Both studies showed immunological changes following perinatal exposure to antibiotics, but weight-related phenotypes were not examined.

Together, these experimental results indicate that early-life antibiotic exposure can have a lasting effect on gut microbiota, host metabolism, weight gain, and adiposity. Interestingly, these effects appear to be exacerbated by a high-fat (“Western”) diet later in life. Moreover, and consistent with some epidemiologic findings, these effects appear to depend on the timing, type, dose, and duration of exposure.

**Conclusions and Directions for Future Research**

While antibiotics provide life-saving treatment for infectious disease, they are frequently prescribed inappropriately, especially to infants and young children [48]. Mounting evidence from epidemiologic and experimental research indicates that antibiotic exposure during critical periods of early de-
velopment may influence weight gain and the development of obesity. Even with the modest effect sizes reported in some studies, these exposures could have a meaningful impact at the population level given the widespread use of antibiotics.

Results from both human and animal studies suggest that gut microbiota play a key role in the apparent association of antibiotics and obesity (Fig. 1), but further research is needed to confirm and characterize the causal mechanisms involved. For example, it will be necessary to isolate the specific microbes and microbial metabolites that influence weight gain in order to identify therapeutic targets and design effective microbiota-based intervention strategies. It is also important to recognize and study the microbiota-independent effects of antibiotics [49], and to explore the potential impact of infections (i.e., the indication for antibiotic treatment) on gut microbiota and weight gain.

Obesity is clearly a complex and multifactorial condition; thus, a multi-pronged prevention strategy will be required to curb the obesity epidemic. Based on current evidence, this strategy should include the judicious use of antibiotics, especially in early life when the developing gut microbiota is particularly susceptible to perturbations with long-lasting implications for metabolic programming and obesity risk.

Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the contents of the chapter.

References
