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Gut microbiota as a potential mediator

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To my children

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ABSTRACT

Childhood growth is a sensitive indicator of health and development, shaped by both biological and environmental factors. Early-life exposures, such as neonatal antibiotic treatment and socioeconomic disadvantage, may influence growth trajectories through complex mechanisms. The gut microbiota, which develops rapidly during infancy, has been proposed as a key mediator linking early exposures to long-term outcomes such as overweight and obesity.

This study examined the associations between neonatal antibiotic exposure, neighborhood socioeconomic status, and childhood growth, and explored the mediating role of the gut microbiota. These associations were studied in the population-based Southwest Finland Birth Cohort, consisting of 14946 children. Growth data were collected from municipal well-baby clinics, and antibiotic exposure was assessed from hospital and prescription records. Neighborhood socioeconomic disadvantage was derived for national grid-based statistics. The impact of perinatal antibiotic exposure on gut microbiota composition was investigated in a well-defined group of infants using 16S rRNA sequencing, and finally fecal microbiota transplantation (FMT) was performed in germ-free mice to explore causality.

We found that neonatal antibiotic exposure was associated with reduced weight and height gain during the first six years of life in boys, while later antibiotic use correlated with increased BMI in both sexes. Socioeconomic disadvantage predicted higher BMI trajectories from age four. Gut microbiota analyses revealed persistent alterations, particularly reduced Bifidobacterium abundance, following neonatal antibiotic exposure. FMT from antibiotic-exposed infants led to impaired growth in male mice, supporting a causal role for microbiota alterations.

These findings suggest that neonatal antibiotic exposure and living in a neighborhood with socioeconomic disadvantage are linked to altered growth patterns in childhood. The gut microbiota is a potential causal mediator of growth disturbances, highlighting its role in early-life health programming. Prudent antibiotic use and addressing social determinants of health may provide avenues for improving child health.

KEYWORDS: childhood growth, antibiotics, gut microbiota, early exposures

TURUN YLIOPISTO
Lääketieteellinen Tiedekunta
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TIIVISTELMÄ

Lapsuuden kasvu on herkkä terveyden ja kehityksen mittari, johon vaikuttavat sekä biologiset että ympäristötekijät. Varhaiselämän altisteiden, kuten vastasyntyneisyyskauden antibioottihoidon ja sosioekonomisen aseman, on osoitettu vaikuttavan kasvun malliin. Suolistomikrobisto kehittyy nopeasti varhaislapsuudessa, ja sen onkin esitetty olevan välittävä tekijä näiden altisteiden ja pitkäaikaisten terveysvaikutusten, kuten ylipainon ja lihavuuden, välillä.

Tutkimuksessa tarkasteltiin vastasyntyneisyyskauden antibioottialtistuksen, asuinalueen sosioekonomisen aseman ja lapsuuden kasvun välisiä yhteyksiä sekä suolistomikrobiston mahdollista välittävää roolia. Aineisto perustui vuosina 2008–10 syntyneistä 14 946 lapsesta muodostettuun Varsinais-Suomen syntymäkohorttiin. Kasvutiedot kerättiin lastenneuvoloista, ja antibioottialtistus määritettiin potilastietojärjestelmistä ja rekisteritiedoista. Asuinalueen sosioekonominen aseman määrittämiseksi tähän rekisteridataan yhdistettiin Ruututietokannan tietoja. Varhaisen antibioottialtistuksen vaikutuksia suolistomikrobistoon tutkittiin aiempaan tutkimukseen rekrytoidusta ryhmästä lapsia. Suolistomikrobistoa analysoitiin 16S rRNA -sekvensoinnilla, ja syy-yhteyden tutkimiseksi antibiootille altistuneiden lasten ulostenäytteitä siirrettiin mikrobittomille hiirille.

Havaitsimme, että vastasyntyneenä saatu mikrobilääkitys liittyi poikien heikompaan painon ja pituuden kasvuun kuuden ensimmäisen elinvuoden aikana, kun taas myöhempi antibioottien käyttö yhdistyi korkeampaan painoindeksiin molemmilla sukupuolilla. Alhainen sosioekonominen asema ennusti korkeampaa painoindeksiä neljän vuoden iästä alkaen. Mikrobistoanalyyseissa todettiin vastasyntyneisyyskauden antibioottihoidon aiheuttavan pitkäkestoisia muutoksia, erityisesti bifidobakteerien vähentymistä. Mikrobistonsiirto antibiooteille altistuneilta lapsilta johti heikentyneeseen kasvuun uroshiirillä, mikä viittaa kausaaliseen syy-yhteyteen suolistomikrobiston ja kasvuhäiriön välillä.

Tulokset osoittavat, että vastasyntyneisyyskauden antibioottialtistus ja asuinalueen sosioekonominen asema ovat yhteydessä kasvun mallin muutoksiin lapsuudessa. Suolistomikrobisto saattaa olla keskeinen välittäjä terveyden ohjelmoitumisessa varhaislapsuudessa. Antibioottien harkittu käyttö ja sosiaalisten terveyserojen huomioiminen voivat tarjota keinoja terveyden edistämiseen.

AVAINSANAT: kasvu, antibiootit, suolistomikrobisto, varhaiset altisteet

Table of Contents

Abbreviations

IAT	intrapartum antibiotic treatment
BMI	body mass index
CS	cesarean section
VD	vaginal delivery
PCoA	principal coordinate analysis
FMT	fecal microbiota transplantation
GF	germ-free
WHO	World Health Organization
SD	standard deviation

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Uzan-Yulzari A*, Turta O*, Belogolovski A, Ziv O, Kunz C, Perschbacher S, Neuman H, Pasolli E, Oz A, Ben-Amram H, Kumar H, Ollila H, Kaljonen A, Isolauri E, Salminen S, Lagström H, Segata N, Sharon I, Louzoun Y, Ensenauer R, Rautava S, Koren O. Neonatal antibiotic exposure impairs child growth during the first six years of life by perturbing intestinal microbial colonization. Nat Commun. 2021 Jan 26;12(1):443. doi: 10.1038/s41467-020-20495-4. PMID: 33500411; PMCID: PMC7838415. (*equal contribution)

II Rautava S, Turta O, Vahtera J, Pentti J, Kivimäki M, Pearce J, Kawachi I, Rautava P, Lagström H. Neighborhood Socioeconomic Disadvantage and Childhood Body Mass Index Trajectories From Birth to 7 Years of Age. Epidemiology. 2022 Jan 1;33(1):121-130. doi: 10.1097/EDE.0000000000001420. PMID :34669629; PMCID: PMC8614531.

III Turta O, Selma-Royo M, Kumar H, Collado MC, Isolauri E, Salminen S, Rautava S. Maternal Intrapartum Antibiotic Treatment and Gut Microbiota Development in Healthy Term Infants. Neonatology. 2022;119(1):93-102. doi: 10.1159/000519574. Epub 2021 Nov 22. PMID: 34808634.

1 Introduction

Growth is crucial to normal development. Repeated measurements of length and weight are aimed to detect growth deviations, which might be an indicator of health issues threatening optimal development. The underlying cause of abnormal growth – whether impaired or excessive - often stems from an imbalance between energy intake and expenditure. In fetal life, typical causes of growth deviation include genetic anomalies, structural defects, and maternal-placental dysfunction. After birth, nutrition becomes the central determinant of growth. During infancy, children rely on breast milk or formula, gradually transitioning to purees and eventually to family foods. This period is not only nutritionally significant but also formative in terms of developing attitudes and behaviors toward food and physical activity.

Of particular concern is the increasing prevalence of overweight and obesity, which often track from early childhood into adulthood. These conditions are associated with long-term metabolic consequences and are notoriously difficult to treat once established. Therefore, prevention efforts must begin as early as possible. Recent studies have highlighted differences in gut microbiota composition between individuals with obesity and those of normal weight, suggesting a potential role for the microbiota in metabolic programming.

Earlier studies have focused on the gut microbiota and its alterations in different diseases and states of health. The gut microbiota, referring mainly to the community of microorganisms in the gut, also carries many functional capacities. This microbial community is continuously tested with external stimuli, which shape the microbiota, its richness and diversity as well as relative abundancies of specific bacteria. Disruptions in microbiota composition, often due to antibiotic use, have been associated with deviations in growth patterns.

Birth represents a critical window for microbiota development. Vaginal delivery facilitates colonization by maternal vaginal and intestinal microbes, whereas cesarean section is associated with delayed and altered microbial diversity. Antibiotics, commonly administered during delivery or in the neonatal period, can further disrupt microbial colonization. Epidemiological studies have shown that infants exposed to antibiotics in early life have a higher risk of overweight and obesity, with some evidence suggesting a dose-response relationship.

Emerging research on fecal microbiota transplantation (FMT) provides evidence that gut bacteria can influence growth outcomes. Studies in both humans and animals have shown that gut microbiota from undernourished or obese individuals can transfer respective growth patterns to recipients. These findings underscore the importance of early-life microbial exposures in shaping long-term health.

This thesis aims to explore the determinants of childhood growth, with a particular focus on early environmental and treatment-related exposures. It examines how antibiotic exposure and neighborhood socioeconomic disadvantage in early childhood influence growth trajectories. Furthermore, it investigates the role of the gut microbiota in mediating these associations, by integrating data from a large, population-based birth cohort, detailed microbiota analyses, and experimental animal models. Understanding how early-life exposures influence growth and health throughout childhood is essential for the development of targeted interventions that promote healthy development and reduce long-term disease risk.

2 Review of the Literature

2.1 Childhood growth and health

Monitoring children's growth is crucially important. Assessment of growth, combined with physiological and developmental trajectories, serves as an essential tool for medical professionals to identify deviations from expected norms and launch timely interventions in case of any indication of health concerns or developmental delays. Normal and consistent growth reflects good overall health and sufficient nutritional status.

There are set milestones, not only for anthropometric measurements and biological markers, but also for psychological and emotional markers and cognition. Deviations in early childhood growth have been shown to correlate also with cognitive outcomes: growth faltering at multiple time periods before the age of two was related to poorer Bayley cognitive outcomes (Scharf et al., 2018).

2.1.1 Follow-up methods, importance and aims

Growth is assessed with various parameters, of which weight, length/height and head circumference are recorded at regular follow-up clinic visits. According to Finnish legislation, it is obligatory to all municipalities to organize a minimum of 15 preventive childcare visits during the first 6 years of the child's life, of which 5 are joint visits with physician and public health nurse. The follow-up clinics use standardized methods for the measurement of length/height and weight provided by the Finnish Institute for Health and Welfare. Length of the infant is measured supine up to 2 years of age, after which standing height is recorded. These repeated measures are combined to growth charts forming a distinguished, individual curve. Growth curves serve as an important tool in evaluating individual growth compared to statistical norms.

There are various ways to assess body composition and possible underweight or overweight. In under 2-year-olds, in Finnish growth charts weight and length are combined in weight-for-length, which is expressed in percentual deviation from mean weight of children of the same length and sex. Other options to define body composition are weight-for-age z-scores in under 2-year-olds and ISO-BMI in over

2-year-olds. Z-scores are preferred as they permit clinical tracking of patients whose anthropometric classification lies beyond the measurable limits of the percentile range, as may happen with both undernourished and obese children. In clinical use the percentile charts may be more of use.

2.1.2 Impaired growth and health

Growth can deviate bidirectionally. Growth deceleration is more often seen in developing countries, and the most common underlying reason is malnutrition or inadequate intake of essential nutrients. The 'triple burden of malnutrition' – stunting, wasting and overweight – is being introduced by WHO, and it is threatening children's ability to survive and thrive. The WHO has reported nearly 50% of deaths among under 5-year-olds are linked to undernutrition, and most of these occur in low- and middle-income countries (WHO, 2025). However, health conditions that may compromise adequate growth, such as various chronic illnesses, endocrinologic and genetic disorders and psychological factors, are seen all around the world.

Childhood growth impairment manifests in diverse ways, with implications for etiology, terminology, and management, as more thoroughly described in Table 1 below. *Growth stunting* is a condition characterized by impaired linear growth in children, resulting in a height-for-age that is significantly below the expected standard for their age and sex, as defined by the WHO. *Wasting* refers to acute undernutrition, poor nutrient intake and/or recurrent illness, resulting to weakened immunity, long-term developmental delay and possibly life-threatening conditions.

The term *faltering growth* (previously called *failure to thrive*) is commonly used to describe a slower-than-expected rate of weight gain in children relative to their age and sex. The preference for the term *faltering growth* reflects a recognition that periods of slow growth may simply represent temporary deviations from typical patterns, while the term *failure* carries potentially negative connotations. Different definitions of faltering growth have been applied over time, leading to considerable variation in prevalence estimates within the UK (NICE, 2017).

Table 1. Comparison of the main forms of growth impairment.

	Faltering	**Wasting**	**Stunting**
Definition	Slower-than-expected weight gain in infants/children, *'failure to thrive'*	Acute undernutrition resulting in low weight for height	Chronic malnutrition resulting in short height for age.
Indicator	Significant downward crossing (of ≥2 centile lines) on a growth chart.	Weight-for-height z-score (WHZ) below -2 SD from the WHO growth standards.	Height-for-age z-score (HAZ) below -2 SD from the WHO growth standards.
Primary Cause	Insufficient short-term or acute dietary intake, illness, or feeding issues.	Recent or acute undernutrition	Prolonged (chronic) inadequate nutrition, repeated infections.
Consequences / Impact	Risk of stunting or wasting. Hindered development if unaddressed.	Immediate risk of increased morbidity and mortality.	Impaired cognitive and physical development. Reduced productivity. Risk of chronic diseases.
Reversibility	Potentially reversible with timely diagnosis and intervention.	Reversible with timely diagnosis and intervention.	Difficult, but partially reversible in early childhood with intervention.
Focus of Monitoring	Weight trends over time.	Weigh-for-height	Height-for-age

Although the proportion of children suffering from stunting or wasting has declined during the last decade, yet 150 million children, 23.2 % of all children under 5 globally are affected by stunting and 42.8 million, 6.6 % are affected by wasting (WHO, 2025). Children suffering from wasting or stunting live predominantly in Africa or Asia. These regions have several lower- and middle-income countries. In high-income countries such as Finland undernutrition is much less important a risk factor for compromised health than overweight and obesity. However, several large cohort studies, also from Finland, have revealed stunting as well as low weight gain during infancy to be associated with ischemic heart disease. (Barker et al., 1989; Eriksson et al., 2001; Frankel et al., 1996) Stunting has also been shown to be associated with lower cognitive development in children (Alam et al., 2020; Walker et al., 2007)

2.1.3 Excessive growth and health

On the contrary to the decreasing prevalence of faltering or stunting growth, obesity and overweight have gained pandemic proportions: its prevalence has continued to increase particularly in upper-middle-income and high-income countries. (Di Cesare et al., 2019; UNICEF & WHO, 2023). It also causes significant health and economic burden (Ling et al., 2023; Okunogbe et al., 2022). Obesity is linked to various health issues, including cardiovascular diseases, type 2 diabetes, and musculoskeletal

disorders. It can also contribute to mental health issues, such as depression and anxiety (Carsley et al., 2019). Childhood obesity has been shown to increase the risk of obesity-related health issues later in life. Treatment of obesity is difficult, and obese and overweight children are likely to become adults with obesity (Simmonds et al., 2016; Singh et al., 2008). Overall, prevention of obesity has become an important goal for all health care professionals and organizations.

Nutrition and physical activity

The simple explanation for obesity is excessive energy intake in comparison to energy expenditure. The obesity epidemic may accordingly be explained by changes related to lifestyle which have occurred during the past decades: diet high in fat, carbohydrates, and energy, but low in fiber combined with sedentary behavior. Energy-rich nutrition is easily accessible and often less expensive than healthier options, and at the same time energy consumption to obtain food and everyday exercise has been decreased.

In adulthood, nutritional choices are ultimately one's own, but these choices and habits often trace back to childhood and adolescence, when nutrition was provided by parents, early childhood education, or school cafeterias. Both the quality of the food available and attitudes toward food are rarely innate; rather, they are largely learned.

The impact of nutritional habits is less apparent in neonates, as breast milk and infant formulas are relatively uniform considering energy composition. Only after introducing purees and solid foods the nutritional components become more variable. Interestingly the growth patterns of breastmilk- and formula-fed infants differ, and infant formula feeding has been represented as a risk factor for obesity during infancy, childhood and even adulthood (Baird et al., 2008; Grummer-Strawn & Mei, 2004). These mechanisms are not entirely established, although human milk oligosaccharides may play an important role (Puccio et al., 2017).

Family and inheritance

Overweight and obesity are highly hereditary due to genetic predisposition and learned behavioral traits. However, hereditary factors fail to explain the globally observed increase in the prevalence of obesity. Early studies from 1990's have shown that parental obesity more than doubles the risk of adult obesity among both obese and nonobese children under 10 years of age (Whitaker et al., 1997). Additionally, maternal health before and during pregnancy is associated with offspring obesity and adverse cardiovascular risk factors (Fraser et al., 2010).

It is widely recognized that obesity in general cannot be traced to a monogenic, not even to a polygenic disorder. Although a few monogenic and polygenic obesity disorders have been described (Panera et al., 2022), they cannot explain the magnitude of the obesity epidemic. The hereditability of obesity has been estimated to be as high as 40 to 70% (Loos & Yeo, 2021; McPherson, 2007), suggesting a multifactorial background with interaction between complex genetic and environmental factors.

This transgenerational transmission of obesity may, in part, be due to the regulation of gene expression rather than changes in the DNA base sequence alone. Epigenetic changes are defined as heritable mechanisms that regulate gene expression and act as an additional layer of information beyond the DNA sequence itself, thus changing the way the body reads DNA. The most studied epigenetic mechanism is DNA methylation, where the addition of a methyl group to cytosine may cause transcriptional changes. There are several environmental and dietary factors influencing epigenetics, such as endocrine disruptors (chemical compounds such as pesticides, bisphenol A, phthalates), hypercaloric and high-fat diet and various micro- and macronutrients. Also, physical inactivity, by reducing insulin sensitivity, energy metabolism and mitochondrial function, contributes to epigenetic changes. (Mahmoud, 2022) Interestingly, the hereditary epigenetic profile is also affected by early life stress and childhood maltreatment exposure (Tuulari et al., 2025).

Socioeconomics and environmental factors

Socioeconomic status (SES) is inevitably closely connected with other factors influencing childhood growth, such as access to healthy nutrition, family eating habits, and physical activity patterns.

On an individual level, SES shapes the choices and options available, with potentially limited financial resources often leading to unhealthy diets, reduced access to physical activity, and delayed or inadequate healthcare. These challenges contribute to poorer health outcomes and are further compounded by chronic stress from financial instability and unfavorable living conditions, which heighten the risk of mental health issues including anxiety and depression. Moreover, lower levels of education can diminish health literacy, making it harder to adopt and maintain healthy lifestyle habits. Over time, these interconnected factors increase the risk of chronic illnesses and reinforce cycles of poor health and inequality.

The influence of SES on health extends beyond the individual level to the community. While the association between neighborhood socioeconomic disadvantage and overweight or obesity in adults is well-established, this relationship in children is less thoroughly explored. However, it seems plausible, as

suggested by previous studies, that children are directly and indirectly affected by neighborhood socioeconomic disadvantage through parental habits and behaviors. For example, childhood neighborhood SES disadvantage has been linked to an increased risk of obesity during school age, adolescence, and early adulthood (Juonala et al., 2011).

In addition to socioeconomic factors, maternal characteristics and early-life events also play a role in childhood obesity. Maternal prepregnancy overweight and obesity, as well as cesarean delivery (Mueller et al., 2015; Mueller, Zhang, et al., 2019), have been associated with a higher risk of obesity in children. It is notable, that mothers today are more likely to be overweight or obese, give birth at an older age, and deliver via cesarean section.

Environmental factors, such as the built environment, can also influence the risk of obesity. A well-designed built environment can promote physical activity and thereby protect against the development of overweight and obesity. For instance, a cohort study in the US found that greater walkability in residential neighborhoods was associated with lower risks of high BMI and obesity (Jia et al., 2019).

2.2 Gut microbiota and growth

The development of the gut microbiota is a dynamic process with profound implications for long-term health (Fig 1). This process unfolds as a function of time, influenced by a range of prenatal, perinatal, and early postnatal factors. Gut microbiota has been shown to play a crucial role in various aspects of human health, including digestion, immune function, metabolism, as well as behavior and satiety. Altered or shifted composition of the gut microbiota, also referred to as dysbiosis, is linked to to deviations in growth as well as development of non-communicable diseases.

Observational studies both in adult and child populations have shown that individuals with obesity have a different microbiota composition compared to those who are lean or of normal weight. (Ismail et al., 2011; López-Contreras et al., 2018; Wang et al., 2024) In addition, deviations in the microbiota already during early childhood have been shown to be associated with later growth. (Schei et al., 2020; Stanislawski et al., 2018)

However, the associations and causality between gut microbiota, nutrition, and growth are challenging to discern. Observational studies have been complemented with experimental animal models, which may provide opportunities to explore these relationships.

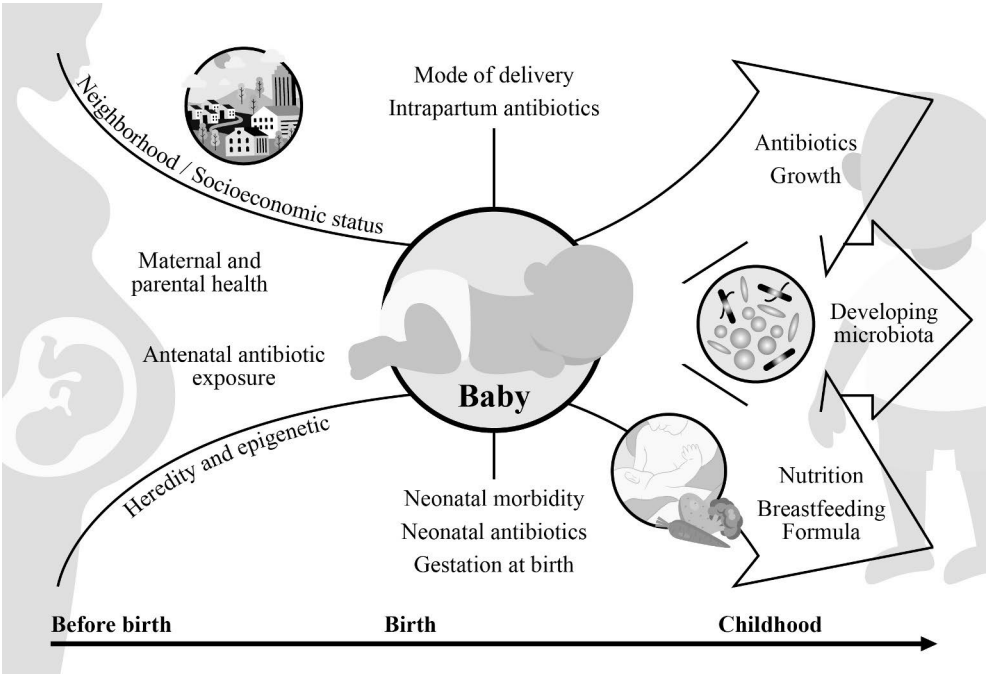


Figure 2. The development of gut microbiota is dynamic, shaped by multiple factors and linked to long-term health.

2.2.1 Development of gut microbiota and its disturbances

Early gut microbiota, particularly during infancy and early childhood, play a crucial role in shaping the long-term composition and function of the microbiota (Vandenplas et al., 2020). It is important to understand how the gut microbiota changes and develops as a function of time in order to comprehend how disturbances occurring at different ages affect the microbiota and, consequently, potentially influence growth.

From birth to early childhood, infants are colonized by and exposed to and a diverse range of microbes, gradually increasing microbial diversity within the individual – a process known as alpha diversity. However, differences in gut microbiota composition between individuals, referred to as beta diversity, are initially high due to different environmental exposures. Over time, these differences decrease as factors such as diet shape the microbiota composition.

The **microbiota** refers to the community of microorganisms – not only bacteria, but also fungi, viruses, and archaea) that live in a specific environment, such as the human gut. It describes the actual organisms present in that ecosystem. The **microbiome**, on the other hand, refers to the collective of these microbes

combined with their genes and their potential to influence the host or ecosystem they inhabit. In this thesis main focus is on the microbiota.

As infants grow, they continuously acquire new microbial taxa, but when comparing across individuals, gut microbiota becomes increasingly similar. The neonatal gut is first colonized by aerotolerant and facultative anaerobes, including lactic acid bacteria (e.g. *Streptococcus, Enterococcus and Lactobacillus*) and *Enterobacteriaceae*. These early colonizers decline rapidly within weeks as breast milk -promoted *Bifidobacteriaceae* increase. As the microbiota matures during infancy and childhood, other anaerobic bacterial families, such as *Bacteroidaeae*, *Lachnospiraceae* and *Ruminococcaceae* become more abundant.

Factors such as mode of delivery (vaginal birth vs. cesarean section), diet (breastfeeding vs. formula feeding), genetics, family and sibling interactions, antibiotic exposure, and environmental influences can all influence the establishment and development of the gut microbiota in early life. (Jakobsson et al., 2014; Penders et al., 2006) (Azad 2013, Rutayisire 2016) In early life, the gut microbiota is relatively unstable and less resilient to disturbances compared to that of adults. However, the gut microbiota diversity and richness as well as the total number of microbes substantially increases during the first few years of life, at which point a more stable, adult-like microbiota is established. Before this stage, the gut microbiota is more susceptible to external influences. (Yassour 2016)

2.2.1.1 Fetal period and prenatal influences

The foundation of gut microbiota development is thought to begin in fetal life. While the sterility of the intrauterine environment, and beginning of the colonization of the gastrointestinal tract, remains debated (Aagaard et al., 2014; Blaser et al., 2021; Briana et al., 2021), emerging evidence suggests that the intrauterine microbiota may influence fetal development (Basak et al., 2022; Kuperman & Koren, 2016). The maternal microbiota is modified by various factors, including diet and metabolic status, already during pregnancy. These prenatal influences contribute to the initial microbial landscape encountered by the infant during and after birth. Disruptions in the mother's gut microbiota can affect the initial bacterial colonization in the newborn, and thus play a key role in the intergenerational transmission of metabolic disease risk.

Gut microbiota composition is linked to weight, and maternal weight gain during pregnancy is affected by microbiota (Collado et al., 2008, 2010; Santacruz et al., 2010). Maternal lifestyle, in particular perinatal exposure to tobacco smoke, affects also significantly on the maternal microbiota (Falara et al., 2024). Even pregnancy itself has been suggested to modify maternal gut microbiota, as various physiological and immunological changes occur during pregnancy (Koren et al., 2012).

Antimicrobial agents cross the placenta, and therefore the microbiota modifying effects of maternal antibiotic use may be transmitted to the developing fetus as well. Antibiotic exposure can have several negative effects on the gut microbiota, such as altered bacterial species diversity and richness, altered metabolic activity, and formation of antibiotic-resistant organisms. (S. Kim et al., 2017; Ramirez et al., 2020) Studies in healthy adults have shown how administration of broad-spectrum antibiotics leads to significant changes in gut microbiota, such as decrease in the prevalence of *Bifidobacterium* and an increase in *Enterobacteriaceae* (Palleja et al., 2018; Panda et al., 2014). The gut microbiota of a healthy adult recovers rather rapidly, within months at these studies, but the alterations in the microbiota of a pregnant mother or a developing fetus and infant may be more long-lasting.

2.2.1.2 Birth as a critical transition

Delivery represents a pivotal moment in the establishment of the gut microbiota. Vaginal delivery (VD) exposes the neonate to the maternal vaginal and gut microbiota, which include beneficial bacterial genera such as *Bacteroides*. (Bäckhed et al., 2015; Reyman et al., 2019) By contrast, infants delivered via cesarean section (CS) are primarily colonized by skin-associated microbiota (Bäckhed et al., 2015; Dominguez-Bello et al., 2010; Stokholm et al., 2016), resulting in delayed microbial diversity and altered composition. These early differences persist for years, with some studies identifying effects on gut microbiota composition up to seven years of age. (Salminen et al., 2004)

A substantial percentage, 20-45% of neonates are exposed to intrapartum antimicrobial agents, mostly due to prophylaxis for maternal group B Streptococcus (GBS) colonization or infection prophylaxis in CS. (Hakkola et al., 2024; Koebnick et al., 2021; Persaud et al., 2015) Although the timing of the prophylaxis is set in a way to minimize the effects to the neonate, it still may cause alterations in the infant microbiota or even clinical effects. (Leppälehto et al., 2018) Maternal intrapartum antibiotic therapy (IAT), although not directly affecting the fetus or neonate, has been shown to cause disruptions in the developing gut microbiota. These effects seem evident, but the duration of microbiota deviations is unclear (Aloisio et al., 2014; Corvaglia et al., 2016; Mazzola et al., 2016; Nogacka et al., 2017; Tapiainen et al., 2019)

2.2.1.3 Neonatal period: postnatal modifiers of microbiota development

In the neonatal period, factors such as feeding practices, antibiotic use, and environmental exposures exert significant influence over microbial colonization (Bogaert et al., 2023). Breastfeeding, for instance, promotes the dominance of

Bifidobacteria and other beneficial microbes through the provision of human milk oligosaccharides (HMOs). Formula feeding, by contrast, results in distinct microbial patterns that may lack these specific benefits. (Davis et al., 2022), and the maturation of gut microbiota towards a more adult-like on is more rapid in formula-fed than breast-fed infants. (Odiase et al., 2023)

Breastfeeding duration is reportedly shorter for mothers with obesity than for normal-weight mothers (Grube et al., 2016; Shipp et al., 2024), which in turn leads to earlier consumption of formula. Breast milk composition has also been shown to differ between obese and normal-weight mothers. (Atanassov et al., 2019; Simon Sarkadi et al., 2022).

A significant number of neonates are exposed to antimicrobials, and often without the evidence of infection. (Persaud et al., 2015) According to current AAP and NICE guidelines (NICE, 2024; Pantell et al., 2021), suspicion of bacterial infection in a neonate leads to initiation of empirical antimicrobial treatment, as sepsis is an important cause of neonatal mortality and morbidity. It is notable, that the incidence of culture-positive sepsis is under 1/1000 live births in high-income countries. (Fleischmann et al., 2021; Giannoni et al., 2022; Gyllensvärd et al., 2024; van Veen et al., 2024) However, the symptoms, signs and early laboratory findings of bacterial sepsis are unspecific and variable, and therefore the threshold to start antibiotic treatment must be low. Approximately 1.9-3.8% of all neonates are exposed to empirical antibiotic therapy (Gyllensvärd et al., 2024; THL, 2024), the majority of which may be discontinued after 36-48 hours when infection has been ruled out and only those with positive or clinically diagnosed sepsis receive a full course. Nevertheless, neonatal antibiotic treatment causes as of yet incompletely described deviations in the developing gut microbiota (Tanaka et al., 2009)

The duration of the gut microbiota alteration caused by antimicrobials is still not clear. In adults, the restoration of gut microbiota to a steady state is thought to be relatively rapid, but less is known about the impact in children. Small study of nine full-term neonates treated with ampicillin and gentamicin found that antibiotic exposure was linked to an increase in fecal *Proteobacteria* and a decrease in *Actinobacteria*, particularly *Bifidobacterium* species, at four weeks of age compared to non-exposed infants. While the abundance of *Actinobacteria* recovered by eight weeks, the higher levels of *Proteobacteria* persisted. (Fouhy et al., 2012) Similar findings were reported by Arboleya and colleagues, who observed that both intrapartum and neonatal antibiotic exposure led to an increased presence of fecal *Enterobacteriaceae* during the first three months of life. (Arboleya et al., 2015)

Premature birth represents a unique challenge to gut microbiota development. Preterm infants often experience limited exposure to maternal microbiota due to early CS delivery, delayed skin-to-skin contact, and postponed initiation of breastfeeding. These infants are also more likely to receive antibiotics, further

disrupting normal microbial colonization. (Tapiainen et al., 2019; Zwittink et al., 2018) In preterm infants, empirical antibiotic therapy administered within the first week of life due to suspected infection was associated with a higher relative abundance of *Enterobacterium* species and reduced gut microbiota diversity by three weeks of age. (Greenwood et al., 2014) While the clinical significance of the overrepresentation of *Proteobacteria* and *Enterobacterium* species is not fully understood, strong evidence suggests that early-life antibiotic exposure significantly increases the risk of overweight and obesity later in life.

2.2.1.4 Infancy and early childhood: dynamic changes of gut microbiota

After the neonatal period, gut microbiota diversity and richness increase substantially during the first three years of life, reflecting the rapid colonization and maturation of the gut ecosystem. (Yatsunenko et al., 2012) Environmental factors such as antibiotic exposure, diet, and host genetics modulate this trajectory.

During infancy the gradual termination of breastfeeding starts simultaneously with the introduction of solid foods. During this weaning period, dietary needs may be complemented with progressive increase of solid foods or formula. Solid food introduction modifies the gut microbiota composition and dictates the beginning of microbiota maturation. (Laursen et al., 2017) The high abundancy of *Bifidobacterium* throughout infancy, and especially during lactation, significantly decreases as infants are weaned and the diet shifts to solid foods. (Arrieta et al., 2014)

The influence of nutrition to gut microbiota in infancy has not been studied extensively, but in a single case study the introduction of table foods lead to development of gut microbiota with characteristic composition and functions of an adult microbiota: increased abundance of Bacteroidetes, enrichment of genes associated with carbohydrate utilization, elevated fecal short chain fatty acid levels and a more stable composition. (Koenig et al., 2011) It is notable, that in infants the interpersonal variation in gut microbiota diversity as well as functional gene content is greater than between adults. (Kurokawa et al., 2007)

Antibiotics in early childhood have been studied largely, and the effects on gut microbiota are clear. Korpela et al (Korpela & de Vos, 2016) have demonstrated the effect of antibiotics on gut microbiota, particularly to Bifidobacterium and Bacteroides.

The relationship between socioeconomic status and gut microbiota has been relatively underexplored. Numerous confounding factors make research in this area challenging. One study in adults from the US found that socioeconomic status accounted for 12–18% of gut microbiota variability. The same study also observed that as socioeconomic status increased, the abundance of *Bacteroidetes* rose while *Prevotella* levels declined. (G. E. Miller et al., 2016)

Overall, the development of the gut microbiota can be visualized as a time-dependent process, with distinct stages shaped by prenatal, perinatal, and postnatal influences. It may be difficult to distinguish the specific actions of all the above-mentioned cofactors since they are closely linked. Maternal overweight and obesity has important effects on the course of pregnancy, delivery, and the offspring. It increases the risk of maternal metabolic disturbances such as gestational diabetes and pre-eclampsia. These in turn are in relation with fetal problems: increased risk of macrosomia/LGA, congenital anomalies, preterm births and stillbirths have been observed. Interventions targeting these critical windows of microbial establishment may offer promising avenues for improving long-term health outcomes.

It is highly challenging to distinguish the individual effects of these factors, as many of them are associated to another. In the Finnish population, there has been not only a significant decline in the number of births but also an increase in the average age of first-time mothers, a decrease in the total fertility rate, a rise in maternal BMI, a higher percentage of obese mothers, and an increase in the number of CS. Similar trends have been observed in other developed countries as well. However, it is reassuring that the achieved reduction in perinatal mortality has not come at the cost of increased neonatal antibiotic treatment; on the contrary, the use of antibiotics has actually declined. (THL, 2024)

2.2.2 Gut microbiota as a mediator of growth

The associations between early-life exposures and overweight and obesity, as well as gut microbiota and growth, seem obvious. Although proceeding from finding associations to proving causality is difficult, for example perturbations of the gut microbiota resulting from antibiotic exposure provide a potential causal mechanism between early antibiotic exposure and altered childhood growth trajectories.

First, the cause must precede the effect. Previous work has shown that gut microbiota is different among obese and lean individuals. (Ley et al., 2005; Turnbaugh et al., 2006) Interestingly, gut microbiota deviations have been shown to precede the development of overweight and obesity. Kalliomäki et al showed that aberrant compositional development of the gut microbiota precedes overweight: specifically, the number of *Bifidobacterium* was shown to be higher during the first year of life in children remaining normal weight at 7 years than in children developing overweight (Kalliomäki et al., 2008). Obesity is associated with changes in the relative abundancies of the *Bacteroidetes* and the *Firmicutes*, and these changes are demonstrated to lead to different capacities to harvest energy from the diet.

Nutrition

Recent advances in scientific research have revealed intriguing mediating factors between the diet, host energy metabolism and the body morphometric phenotype. Namely, the indigenous intestinal microbiota has been suggested to not only be influenced by diet but also to play a causal role in the development of different morphometric types.

The transfer of undernourished phenotype has been studied in two groups with known growth disturbances: undernourished children in low-income countries, as in study by Blanton, and in preterm neonates, as in study by Hiltunen. In Blanton's important study fecal samples from Malawian 6- and 18-month-old children with healthy growth patterns or with various degrees of undernutrition were obtained and transplanted to young germ-free mice. (Blanton et al., 2016) It was shown that not only the undernourished children had immature gut microbiota, but it also transmitted growth impairment and metabolic abnormalities in tissues to mice after fecal microbiota transplantation (FMT). Interestingly, microbiota from the 6-month-old infant produced greater effect on growth than microbiota from 18-month-old infants. Similar type of underdevelopment of the gut microbiota has also been shown in *failure to thrive* infants (Zhang et al., 2022).

In the study by Hiltunen et al, FMT to germ-free mice was conducted with the meconium of very preterm (<32 weeks), moderately pre-term (32-37 weeks) and term (>37 weeks) neonates. Findings suggested that FMT of very preterm neonate gut microbiota results in impaired growth, altered metabolic parameters and intestinal immune function typical for very preterm infants, in the contrast to FMT with meconium from term neonates. (Hiltunen et al., 2021) These studies are amongst few to provide evidence to causality between gut microbiota deviations and growth.

Mode of delivery

Epidemiological studies have shown that the mode of delivery has a significant impact on the development of the newborn's gut microbiota, as discussed in 2.2.1.2. CS has been associated with modestly higher risks of overweight and obesity in childhood (Flemming et al., 2013; H. Li et al., 2013), which may partly reflect disruptions in early microbial colonization. Interestingly, rising CS rates have

paralleled increasing maternal body mass index (BMI), which is also seen in Finnish register data (THL, 2024)(Fig. 2 below).

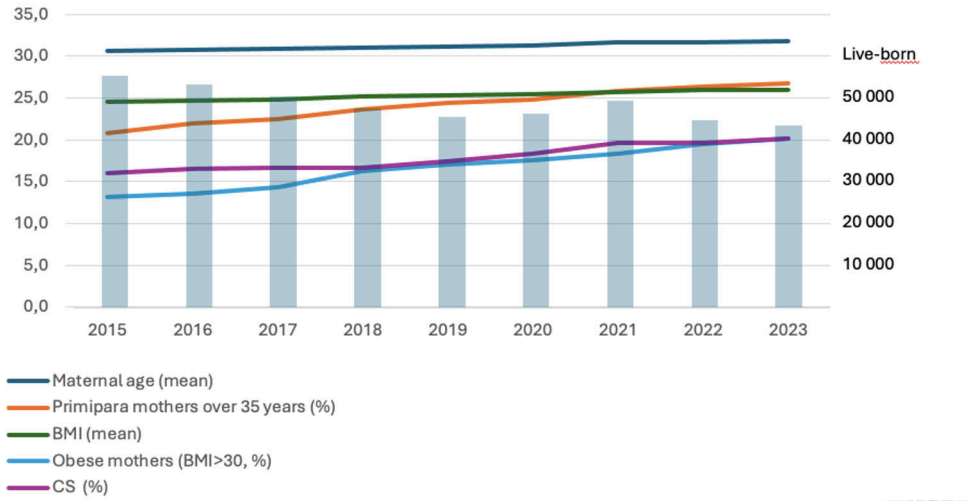


Figure 2. Rising trends of cesarean section rates, maternal age, and maternal obesity in Finland.

In addition, obesity is a recognized risk factor for elective and emergency CS (Ellekjaer et al., 2017; Poobalan et al., 2009). It is still unclear, whether this reported association of CS and obesity in the offspring is simply an indicator of maternal obesity, as maternal pre-pregnancy weight serves as an important confounder. Nevertheless, CS has been suggested to be an independent risk factor for increased risk of offspring overweight and obesity after adjusting for maternal pre-pregnancy weight. (Kuhle et al., 2015)

The increasing rates of cesarean delivery in developed countries - driven by both medical and non-medical factors - raise concerns about its impact on microbial development. Added to this, as international clinical guidelines recommend administration of prophylactic antibiotics prior to surgical incision in CS to reduce maternal infectious morbidity and mortality around the time of birth, the assessment without confounding factors is difficult. Martinez et al performed an animal study with antibiotic-free, fostered C-sections to evaluate the impact of mode of delivery on the early microbiota and body weight during development. In this experimental study, researchers found that mice delivered via C-section gained more body mass after weaning and did not exhibit the dynamic developmental changes in gut microbiota seen in the control group. This effect was more pronounced in females. The results indicate a causal link between C-section and increased body weight, highlighting the role of maternal vaginal bacteria in normal metabolic development. (Martinez et al., 2017)

As noted, studies have shown that being born via CS is associated with an increased risk of inflammatory and metabolic diseases, and CS also appears to be associated with short-term but observable changes in the neonate's gut microbiota. Dominguez-Bello and colleagues suggested that correcting this microbiota disturbance could help prevent later disease, proposing the transfer of the mother's vaginal microbiota to the newborn after birth, a procedure known as '*vaginal seeding*'. (Dominguez-Bello et al., 2016) The pilot study comprised four neonates, and the researchers could show a partial restoration of infant microbiota after vaginal seeding. Introduction of this adventurous procedure led to discussion of the balance between benefits and risks, as well as the potential clinical use. (Clemente & Dominguez-Bello, 2016; Cunnington et al., 2016; Lokugamage, 2016; Mueller, Hourigan, et al., 2019) Although the concept is compelling, there is insufficient data to support its widespread adoption. Ongoing clinical trials are aimed to assess obesity and atopy outcomes after vaginal seeding. (Hourigan et al., 2025) In addition, as early colonizers of the neonatal microbiota derive largely from maternal fecal microbiota, maternal FMT to neonates has also been suggested. In a proof-of-concept study of 7 mother-neonate-pairs, restoration of gut microbiota of CS-born infants was demonstrated. (Korpela et al., 2020)

Antibiotics

The strongest evidence of the association between antibiotic exposure and growth rises from a number of well-conducted epidemiological studies suggesting that antibiotic exposure **in infancy** is associated with a higher BMI and increased risk of overweight and obesity in childhood. Exposure to antibiotics appears to be particularly detrimental during the first six months of life (Ajslev et al., 2011; Azad et al., 2014; Bailey et al., 2014; Saari et al., 2015; Trasande et al., 2013)

Antibiotic exposure during the first six months of life was associated with overweight at the age of two years in boys (adjusted OR 1.34 with 95 % CI 1.06–1.66) in a cohort study of 12,000 children from Finland. This association was not seen in girls (adjusted OR 1.16 with 95 % CI 0.87–1.56). (Saari et al., 2015) In an epidemiological study of more than 11,000 children from the United Kingdom, antibiotic exposure during the first 6 months of age significantly increased the risk of overweight at 38 months of age (OR 1.22 after adjusting for potential confounding factors; $p = 0.029$). Exposure to antibiotics at 6 to 14 months of age was not associated with BMI; however, exposure to 15 to 23 months was significantly associated with increased BMI z-score at 7 years of age. (Trasande et al., 2013)

Moreover, a dose-response relationship was identified between the number of antibiotic courses administered in the first two years of life and the risk of childhood obesity in a cohort of over 64,000 children from the United States. (Bailey et al.,

2014) These large-scale epidemiological studies, involving tens of thousands of participants, provide strong evidence of an association between early antibiotic exposure and the development of overweight. The observed dose-response effect may suggest a causal relationship.

Already decades ago, large number of farm animals were fed low-dose antibiotics as it was noted to promote weight gain. The finding that subtherapeutic antibiotic exposure can promote weight gain is particularly significant, as antibiotics are commonly used in livestock to enhance growth in many countries, potentially exposing humans to low-dose antibiotics through meat consumption. It is notable, that the European Union has banned many low-dose antimicrobial uses since the 2000s. (Sorensen et al., 2014)

Research from Dr. Blaser's group has shown that administering low-dose antibiotics from weaning leads to greater fat mass accumulation in mice (Cho et al., 2012). This effect appears to be driven by antibiotic-induced alterations in gut microbiota, which enhance short-chain fatty acid production and disrupt host metabolism, including the upregulation of genes involved in fat synthesis. In addition, maternal exposure to low-dose penicillin during late gestation and lactation had a more pronounced impact on weight and fat accumulation than direct exposure after weaning. (Cox et al., 2014) These results strongly suggest a critical time window in which exposures may be particularly detrimental.

In addition, male mice were more profoundly affected than females by perinatal antibiotic exposure, which is consistent with the epidemiological data showing increased risk of overweight and obesity in antibiotic-exposed boys discussed above.

The impact of **perinatal or prenatal antibiotic exposure** on growth is not as clear as neonatal or early childhood antibiotics. Antimicrobial agents cross the placenta and therefore could have an impact on fetus, its microbiota and eventually growth. The association of antibiotic exposure during pregnancy and birth weight has been examined in a number of studies, but sample sizes have been variable, and no consensus can be made. (Luntamo et al., 2013; Mueller et al., 2017; Vidal et al., 2013; Zhang et al., 2019) In a Chinese cohort of 2543 preterm infants, exposure to antibiotics during pregnancy was associated with higher birth weight z-scores (Luo 2021) On the other hand, in a US cohort (Metz et al., 2020) exposure to intrapartum antibiotic prophylaxis was not associated with higher BMI z-scores in early childhood, as evaluated based on the first available BMI z-score at 2-5 years of age. In a Danish cohort study of 63 300 pregnant women prenatal antibiotic exposure was not consistently associated with birth weight, although in stratified analyses boys born preterm were significantly heavier at birth after antibiotic exposure. (Tomar et al., 2022)

The **long-term** effects of perinatal antibiotic exposure on growth have not been conclusively evaluated. Nonetheless, in a cohort study from Shanghai, the association between cumulative antibiotic exposure during pregnancy and infant growth was examined in 295 mother-child pairs (Zhou et al., 2021) and the researchers found a significant positive association between antibiotic exposure and growth speed from 2 to 6 months. In addition, exposure to antibiotics in the second or third trimester was positively associated with BMI z-scores up to 7 years of age in 436 mother-child dyads (Mueller et al., 2015). Large cohort studies examining intrapartum antibiotic prophylaxis and childhood weight gain from the research collaboration of Puopolo and Schrag found that GBS-specific IAP was associated with a modest increase in rate of weight gain in the first 5 years of age (Koebnick et al., 2021; Mukhopadhyay et al., 2021).

The strength of association would be expected to rise when considering early **neonatal antibiotic** exposure and growth, but only limited data are available. In an observational study from the Netherlands (Kamphorst et al., 2019) antibiotic exposure in the first days of life was associated with reduced growth during the first year of life in a study of 436 term infants. Interestingly, the same study reported antibiotic use after the neonatal period to be linked with excessive childhood weight gain particularly in boys.

3 Aims

This thesis examines the determinants of childhood growth, particularly the early environmental and iatrogenic, treatment-related factors. The specific aims of the study are listed below.

1. To investigate the association of early exposures on childhood growth, specifically
    a. Neonatal antibiotics (Original publication I), and
    b. Neighborhood socioeconomical status (Original publication II)
2. To scrutinize the impact of antibiotic exposure on gut microbiota
    a. In perinatal period (Original publication III)
    b. In immediate neonatal period (Original publication I)
3. To examine the potential causal role of the gut microbiota in the mediating association between early antibiotic exposure and childhood growth patterns (Original publications I)

The composite hypothesis was that the gut microbiota acts as an intermediary factor between early environmental determinants and growth, and the short-term, early-life exposures have long-lasting effects.

4 Materials and Methods

4.1 Early exposures and childhood growth (I, II)

4.1.1 Materials

The association between early exposures and childhood growth (**Original Studies I and II**) was studied in the population-based Southwest Finland Birth Cohort (SFBC). At the time of the studies, the Hospital District of Southwest Finland included two maternity hospitals, and there were no other private or municipal maternity hospitals in the area. All children born in the geographical area of Southwest Finland during the 3-year period of 2008-2010 were included in the cohort, and consequently the SFBC cohort consists altogether 14 946 children.

To ensure the independence of the study subjects, only the first child from each mother during the study period was included in these studies. This also meant excluding children born from multiple gestation pregnancies. Children with chronic disease affecting growth such as genetic syndromes, significant congenital heart disease, malignancies or endocrine, and growth disturbances requiring growth hormone therapy were also excluded.

In **Study I**, some additional exclusion criteria were used. Children born preterm (i.e. gestational age $36^{6/7}$ weeks or less) were excluded due to significantly increased risk of both perinatal antibiotic exposure and impaired growth. Altogether 12 422 children were included in **Study I** (Figure 3).

In **Study II**, subjects with missing information on height or weight at birth, no growth measurements between ages 1 and 7 or missing information on neighborhood socioeconomic disadvantage were excluded, leaving 11 023 children in the analytic study population (Figure 3).

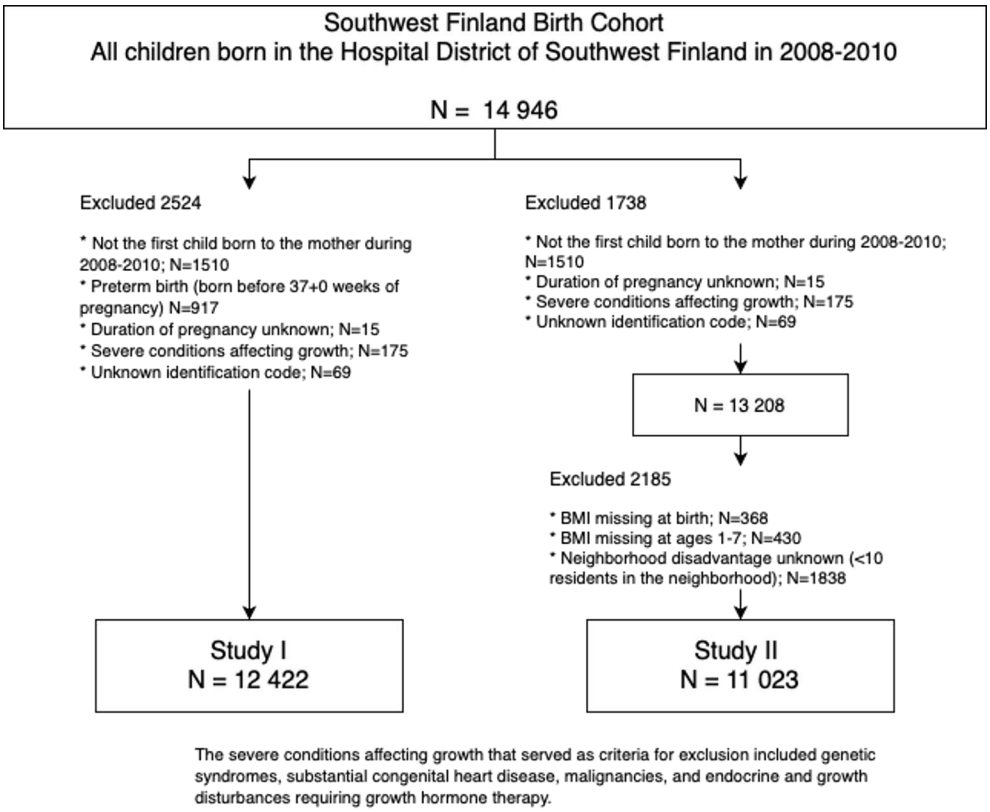


Figure 3. Study population.

Data regarding the pre- and perinatal characteristics of the SFBC study subjects and their mothers including gestational age; birth weight and length; sex; mode of delivery (vaginal or cesarean section); maternal age; maternal prepregnancy weight, height and BMI; primiparity (no previous deliveries); single parenthood (not married or cohabiting at the time of childbirth); smoking during pregnancy (yes or no); intrapartum antibiotic treatment, time of commencement and duration of antibiotic therapy; gestational diabetes mellitus and other medical conditions the mother manifested with during pregnancy; and maternal profession were extracted from the Medical Birth Register maintained by the Finnish Institute for Health and Welfare. Based on register data on mother's self-reported occupation, parental SES was defined for **Study III** and grouped in 6: higher-grade nonmanual, lower-grade nonmanual, manual, student, full-time mother, or other. The primary language of the mother was obtained from the Population Register Center, and mothers were

classified as having immigrant background if their primary language was not Finnish or Swedish.

Childhood growth data were obtained from municipal well-baby clinics. Children enter the school health care system in the autumn term of the year they turn 7 years of age and, consequently, the municipal well-baby clinic follow-up is completed at the age of 6–7 years.

Data on antibiotic purchases during the first six years of life were extracted from the Drug Prescription Register maintained by the Social Insurance Institution of Finland.

For **Study I**, data regarding neonatal antibiotic exposure and diagnoses of neonatal bacterial infections during the first 14 days of life were extracted from the hospital records. As per the protocol of the neonatal unit at the Turku University Hospital throughout the duration of the study, antibiotic therapy was rapidly initiated in all neonates with symptoms or signs suggesting early-onset sepsis and the initial empirical antibiotic therapy consisted of a combination of intravenous benzylpenicillin and gentamicin. The children in the cohort were grouped as follows: (1) empirical antibiotic therapy, which was discontinued after infection had been ruled out, (2) antibiotic therapy for confirmed or clinical infection, and (3) no neonatal antibiotic exposure.

4.1.2 Methods

4.1.2.1 Neonatal antibiotics and growth (I)

The anthropometric data closest to the time points of 6 months and 1, 2, 3, 4, 5, and 6 years of age were used in the analyses. The Finnish growth charts (Sankilampi et al., 2013) were used to obtain population-specific z-scores for height, weight, and BMI. The association between neonatal antibiotic exposure and weight and height z-scores at 6 months and 1 year and weight, height and BMI z-scores at 2, 3, 4, 5, and 6 years of age were analyzed using hierarchical linear mixed model for repeated measurements using the "MIXED" procedure of SAS, version 9.4 (SAS Institute, Cary, NC, USA).

Gestational age, birth weight z-score, mode of delivery (vaginal or cesarean section delivery), maternal prepregnancy BMI, and intrapartum antibiotic treatment (yes or no) were included in the hierarchical linear mixed model for repeated measurements as explanatory variables with neonatal antibiotic exposure to control for potential confounding factors which might be associated with neonatal antibiotic exposure and affect childhood growth.

Interaction between neonatal antibiotic exposure and time was included in the model to examine whether mean change over time was different between antibiotic

exposure groups. An unstructured covariance pattern was used for repeated measures. Normal distribution assumption was checked from studentized residuals.

Clinical characteristics of the study population are presented in the Table 2 below.

Table 2. Clinical and background characteristics of the study population in the neonatal antibiotic exposure and growth study.

	MALE (N=6307)				FEMALE (N=6115)			
	No AB and no infection	**AB and no infection**	**AB and infection**	**P**	**No AB and no infection**	**AB and no infection**	**AB and infection**	**P**
N	5634 (89.3%)	297 (4.7%)	376 (6.0%)		5637 (92.2%)	216 (3.5%)	262 (4.3%)	
Gestational age (weeks)	40.03 (1.22)	40.11 (1.38)	40.06 (1.34)	0.49	40.10 (1.18)	39.98 (1.45)	40.10 (1.30)	0.34
Birth weight (grams)	3625 (462)	3680 (530)	3754 (499)	<0.0001	3509 (439)	3509 (602)	3614 (512)	0.0011
Birth weight Z score	0.008 (1.03)	0.127 (1.18)	0.293 (1.11)	<0.0001	-0.006 (1.04)	-0.020 (1.43)	0.236 (1.20)	0.0014
Birth height (cm) *	51.3 (2.04)	51.6 (2.19)	51.8 (2.12)	<0.0001	50.4 (1.91)	50.4 (2.24)	50.9 (2.29)	0.0003
Birth height Z score	0.080 (1.07)	0.242 (1.14)	0.332 (1.10)	<0.0001	0.047 (1.04)	0.051 (1.23)	0.335 (1.25)	0.0003
Maternal prepregnancy BMI (mean. SD) *	24.3 (4.74)	25.2 (5.16)	25.2 (5.39)	<0.0001	24.3 (4.72)	25.0 (5.00)	24.9 (4.70)	0.032
Vaginally delivered	87.7% (4941/5634)	82.8% (246/297)	83.2% (313/376)	0.0030	88.0% (4959/5637)	77.0% (166/216)	87.4% (229/262)	<0.0001
Maternal intrapartum antibiotic treatment	10.2% (571/5619)	16.2% (48/296)	16.8% (63/375)	<0.0001	10.5% (588/5614)	9.3% (20/216)	18.3% (48/262)	0.0003
Number of antibiotic drugs up to six years of age**	7 (4, 12)	7 (3, 11)	7 (4, 13)	0.32	6 (3, 10)	6 (3, 11)	7 (3, 11)	0.23
Neonatal antibiotic exposure duration (days)***	-	2 (2, 2)	7 (7, 7)	<0.0001	-	2 (2, 2)	7 (7, 7)	<0.0001
Neonatal antibiotic exposure start age (hours from birth)***	-	5.48 (1.45, 24.87)	4.08 (1.75, 18.52)	0.56	-	3.48 (1.40, 14.10)	3.68 (1.54, 16.35)	0.81

Previous epidemiological (S. A. Miller et al., 2018; Saari et al., 2015) and experimental (Cox et al., 2014) studies suggest that the susceptibility to growth disturbances after early-life antibiotic exposure is sex-specific. In addition, in our cohort boys were significantly more often exposed to neonatal antibiotics, and for these reasons boys and girls were analyzed separately.

4.1.2.2 Childhood antibiotic use and growth (I)

The same approach as described in **4.1.2.1** was used to analyze the association between the number of antibiotic purchases after the neonatal period and child growth during the first six years of life. The models contain the same response variables. The number of antibiotic purchases, neonatal antibiotic exposure, gestational age, birth weight z-score, mode of delivery (vaginal or cesarean section delivery), and maternal prepregnancy body mass index were included in the model as explanatory variables. Background characteristics of the study population are presented in the Table 2 above.

4.1.2.3 Neighborhood socioeconomic status and growth (II)

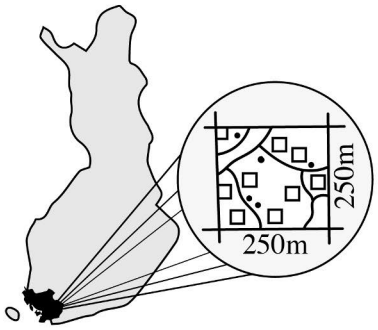
The anthropometric data at birth and closest to the time points of 1 and 2 years of age (within 3 months), and 3, 4, 5, 6, and 7 years of age (within 6 months) were used in the analyses. The World Health Organization growth charts were used to obtain age-specific z-scores for BMI. BMI z-scores +1 SD and +2 SD were used to estimate the prevalence of overweight and obesity, respectively. The numbers of participants with available height and weight measurement data at each time point are presented below (Table 3).

Table 3. The number of participants with available height and weight measurement data.

AGE (YEARS)	TOTAL	BOYS	GIRLS
BIRTH	11,023	5,635	5,388
1	11,023	5,635	5,388
2	10,879	5,569	5,310
3	10,628	5,435	5,193
4	10,360	5,310	5,050
5	9,685	4,950	4,735
6	8,021	4,102	3,919
7	4,715	2,423	2,292

In order to formulate cumulative neighborhood disadvantage, data regarding neighborhood social disadvantage and residential data were needed.

Data for neighborhood social disadvantage were derived from a grid database maintained by Statistics Finland. It contains socioeconomic information from each residence at a spatial resolution of 250 m by 250 m, and the grid data were obtained with 5-year intervals between 1990 and 2015. The neighborhood disadvantage score was formed as described in Figure 4. For the statistical analyses, the neighborhood disadvantage score was classified into four categories based on national means as follows: <–1 SD (lowest disadvantage), –1 to 0 SD, ≥0 to 1 SD, and >1 SD (highest disadvantage).



Z-score based on Finnish population		Neighborhood Disadvantage Score
1. LOW EDUCATION	Z_1	
2. UNEMPLOYMENT RATE	Z_2	Mean (Z_1, Z_2, Z_3)
3. AVERAGE INCOME	Z_3	

Figure 4. The neighborhood disadvantage score was formed based on the proportion of adults with low education, the unemployment rate, and the average annual income of households in each grid area. Missing data (i.e., areas with fewer than 10 residents in the neighborhood) was replaced with the mean neighborhood disadvantage score of the eight adjacent map squares. For each of the three variables, a standardized z-score was derived based on the total Finnish population (mean = 0, SD = 1). A score for neighborhood disadvantage was then calculated by taking the mean value across the three z-scores. Higher scores on the continuous index denote greater disadvantage. For the statistical analyses, the neighborhood disadvantage score was classified into four categories based on national means as follows: <–1 SD (lowest disadvantage), –1 to 0 SD, ≥0 to 1 SD, and >1 SD (highest disadvantage).

Residential mobility data, which was based on a complete history of the residential addresses with latitude and longitude coordinates, was obtained from the Population Register Center for each mother and her child until the child was 7 years old. Using open-source Geographical Information Systems (QGIS, http://www.qgis.org/en/site/), data on the cumulative residential neighborhood disadvantage for each time point were linked to the cohort participants' home addresses by the latitude and longitude coordinates. Time-dependent socioeconomic disadvantage score weighted by residential time at each location was calculated for each study subject.

Missing information for binary confounders was imputed using the mode value. With the repeated measured outcome and exposure missing data in between birth and the last measurement were imputed using the mean of observed values of the person. Occupational status data were missing for 4% of participants; these participants were assigned to '*other'* category. To examine the associations of the pre- and perinatal characteristics with the neighborhood disadvantage categories at birth, the chi square test for categorical variables and general linear model for continuous variables was used. The same models were used to examine the associations of potential confounders with BMI z-score at birth and at the last measurement.

To model the trajectories of childhood BMI until school age, marginal structural models with generalized estimating equations (GEE) and inverse probability weighting were used. For weighted estimation of the parameters in the marginal structural models three models were fitted: the exposure model, the censoring model, and the structural (i.e., weighted) model, as described in Study III.

At birth, the participants lived in 3,791 different neighborhoods (mean population density 86). Only 13% of the neighborhoods had more than five cohort members. There was only one cohort member in 51% of the neighborhoods. Altogether 6,546 (59%) participants moved to other neighborhoods during the follow-up. Consequently, there was no clustering by neighborhood to be corrected in the models. The background and clinical characteristics of the study population in total and by neighborhood disadvantage groups are presented in Table 4.

Table 4. Background and clinical characteristics of the study population in total and by neighborhood disadvantage groups in the neighborhood socioeconomic status and growth study.

		Neighborhood Disadvantage			
	TOTAL	<−1 SD (Lowest) N (%)	−1 to 0 SD N (%)	>0 to 1 SD N (%)	>1 SD (Highest) N (%)
	11023	1412 (13)	5163 (47)	3202 (29)	1246 (11)
Maternal characteristics					
Age (years), mean (SD)	30,0 (5,1)	32,0 (4,3)	30,8 (4,8)	28,8 (5,3)	27,5 (5,6)
Primiparous, N (%)					
No	5523 (50)	874 (62)	2570 (50)	1456 (46)	623 (50)
Yes	5500 (50)	538 (38)	2593 (50)	1746 (55)	623 (50)
Mode of delivery, N (%)					
Vaginal	9539 (86)	1223 (87)	4473 (87)	2752 (86)	1091 (88)
Cesarean section	1484 (14)	189 (13)	690 (13)	450 (14)	155 (12)
Single parenthood at birth, N (%)					
No	10352 (94)	1393 (99)	4974 (96)	2913 (91)	1072 (86)
Yes	671 (6)	19 (1)	189 (4)	289 (9)	174 (14)
Immigrant, N (%)					
No	10054 (91)	1382 (98)	4940 (96)	2864 (89)	868 (70)
Yes	969 (9)	30 (2)	223 (4)	338 (11)	378 (30)
Smoking during pregnancy, N (%)					
No	9762 (89)	1361 (96)	4733 (92)	2710 (85)	958 (77)
Yes	1261 (11)	51 (4)	430 (8)	492 (15)	288 (23)
Obesity before pregnancy (BMI > 30), N (%)					
No	9773 (89)	1289 (91)	4607 (89)	2806 (88)	1071 (86)
Yes	1228 (11)	123 (9)	556 (11)	396 (12)	175 (14)
Gestational diabetes mellitus, N (%)					
No	9332 (85)	1226 (87)	4359 (84)	2700 (84)	1047 (84)
Yes	1691 (15)	186 (13)	804 (16)	502 (16)	199 (16)
Other medical conditions,c N (%)					
No	10690 (97)	1373 (97)	5019 (97)	3106 (97)	1192 (96)
Yes	333 (3)	39 (3)	144 (3)	96 (3)	54 (4)
Parental socioeconomic status, N (%)					
Higher-grade nonmanual	2297 (21)	458 (32)	1265 (25)	476 (15)	98 (8)
Lower-grade nonmanual	2203 (20)	385 (27)	1150 (22)	548 (17)	120 (10)
Manual	3234 (29)	300 (21)	1418 (28)	1082 (34)	434 (35)
Student	1206 (11)	63 (5)	504 (10)	443 (14)	196 (16)
Full-time mother	465 (4)	25 (2)	132 (3)	161 (5)	147 (12)
Other	1618 (15)	181 (13)	694 (13)	492 (15)	251 (20)
Child characteristics					
Sex of the child, N (%)					
Boy	5635 (51)	739 (52)	2631 (51)	1657 (52)	608 (49)
Girl	5388 (49)	673 (48)	2532 (49)	1545 (48)	638 (51)
Preterm birth, N (%)					
No	10566 (96)	1362 (96)	4940 (96)	3076 (96)	1188 (95)
Yes	457 (4)	50 (4)	223 (4)	126 (3)	58 (5)
Duration of pregnancy (weeks), mean (SD)	39,9 (1,5)	39,8 (1,5)	39,9 (1,5)	39,9 (1,5)	39,8 (1,6)
Birth weight (g), mean (SD)	3527 (506)	3571 (495)	3536 (507)	3510 (507)	3482 (511)

With the marginal structural models including the age-disadvantage interaction term, the mean level of BMI z-score and the 95% confidence intervals (CI) at each age by the categories of cumulative neighborhood disadvantage from birth onward were estimated. Sex differences in the BMI trajectories were tested in a model including the interaction term "sex*age* cumulative neighborhood disadvantage." As there was no interaction (P = 0.54), the results are shown for boys and girls combined. The fully adjusted model controlled for child sex and preterm birth, maternal risk factors, and parental SES. Using contrast, the mean difference between categories of cumulative neighborhood disadvantage at each age was calculated using the lowest category of disadvantage as a reference group. These analyses were replicated using the continuous disadvantage score as the measure of exposure at each age. The changes in BMI z-score were also estimated within each category of neighborhood disadvantage level in three different age periods: from birth to age 1 year, from 1 to 4 years, and from 4 to 7 years.

As a sensitivity analysis, the observed trajectories of BMI z-score were calculated according to the individual components of the neighborhood disadvantage score: educational level, unemployment rate and average household income in the neighborhood. Additional analyses were as well performed using the alternative cutoffs of 0.5 SD and 1.5 SD to examine whether the findings were sensitive to specific cutoffs.

Finally, the risk of overweight or obesity at 6–7 years of age was examined by the level of cumulative neighborhood disadvantage. All children who completed the follow-up were included for these Poisson regression analyses, and the models were adjusted for child sex and preterm birth, maternal risk factors, and parental SES. The results are expressed as risk across the categories of neighborhood disadvantage and the risk ratios, and their 95% CIs compared with the lowest disadvantage category. Sex differences in the associations were tested in a model including the interaction term "sex*cumulative neighborhood disadvantage." As there were no interactions (test for overweight P = 0.43 and for obesity P = 0.43), the results are shown for boys and girls combined. All analyses were performed using the SAS software version 9.4 (SAS Institute Inc., Cary, NC).

4.2 Impact of antibiotic exposure on gut microbiota (I, III)

4.2.1 Materials

Perinatal antibiotic exposure and its effects on developing gut microbiota (**Studies I and III**) was investigated in subjects originally recruited to a clinical maternal probiotic intervention trial (Rautava et al., 2012). The original study included pregnant women with atopic sensitization and either a history of or active allergic disease and the intention to breast feed for a minimum of 2 months, and its aim was to study the effects of maternal antenatal and postnatal probiotic use on occurrence of atopic eczema in children. Maternal allergic disease was assessed by patient-reported clinical history of atopic eczema, allergic rhinoconjunctivitis, food allergy, or asthma. Sensitization was verified by skin prick testing. Altogether 241 mothers were randomized to receive either 1) Lactobacillus rhamnosus LPR and Bifidobacterium longum BL 999, 2) Lactobacillus paracasei ST11 and Bifidobacterium longum BL 999, or 3) placebo from 2 months before expected delivery and during breastfeeding until the child was 2 months of age.

To examine the association of **intrapartum antibiotic exposure** on gut microbiota (**Study III**), 24 infants who were exposed to IAT were identified from this original study population. The control group consisted of 24 infants from the same study population who were not exposed to IAT. Controls were matched first by maternal intervention group and second by duration of breastfeeding. All subjects were born by vaginal delivery at full term (after 37 weeks of gestation) and breastfed beyond the age of 1 month (Table 5). Subjects with significant congenital anomalies were excluded from the study. None of the infants included in **Study III** received antibiotics during the immediate neonatal period.

	IAT (n=24)	**no IAT (n=24)**	**P**
Gestational age (weeks)	39.8 (37.6 – 41.9)	39.6 (37.6 – 42.3)	0.61
Boys	17 (71%)	11 (46%)	0.08
Birth weight (grams)	3434 (2850 – 4100)	3458 (2430 – 4220)	0.85
Breastfed at 1 month of age	24 (100%)	24 (100%)	*NA*
Breastfed at 6 months of age	16 (66.7%)	18 (75%)	0.53
Maternal prepregnancy BMI (mean, SD)	24.6 (18.4 – 33.6)	23.4 (19.7 – 38.5)	0.37
Maternal age (mean, SD)	35 (25 – 43)	34 (28 – 44)	0.31

Table 5. Clinical characteristics of the infants exposed (IAT) and not exposed (no IAT) to intrapartum antibiotic treatment in Study III. The data are presented as means (range) for continuous variables and as numbers (%) for categorical variables. Student's t-test for independent samples was used for continuous variables and Chi-squared test for categorical variables. NA = not applicable.

In **Study I**, **neonatal antibiotic exposure** and gut microbiota changes were studied from the same original study population in 13 neonates who were exposed to antibiotic therapy with intravenous benzylpenicillin and gentamicin during the first 48h of life. Altogether 20 full-term healthy neonates from the same study not exposed to antibiotics in the neonatal period were selected as controls. The neonates exposed and not exposed to neonatal antibiotics were similar regarding duration of gestation, birthweight, mode of birth, exposure to antibiotics during delivery or the first six months of life after the neonatal period, and breastfeeding at 1 month of age (Table 6). However, the mean duration of breastfeeding was shorter in the infants exposed to neonatal antibiotics.

To establish potential causality between neonatal antibiotic exposure, gut microbiota perturbations, and growth deviation, fecal microbiota transplantation (FMT) from these 13 antibiotic-exposed and 20 non-exposed infants to germ-free (GF) mice was performed in a manner described below in 4.3.2.

	Neonatal antibiotics (N=13)	**Control (N=20)**	**P**
Gestational age (weeks)	$39^{5/7}$ ($36^{0/7}$ – $42^{1/7}$)	$40^{4/7}$ ($37^{4/7}$ – $42^{0/7}$)	0.17
Birth weight (grams)	3708 (2810 – 4660)	3729 (2930 – 4460)	0.90
Intrapartum antibiotic exposure	38% (5/13)	10% (2/20)	0.052
Vaginal delivery	92% (12/13)	100% (20/20)	0.17
Female	31% (4/13)	45% (9/20)	0.41
5 min Apgar score	7.9 (3-10)	9.0 (8-10)	0.10
Breastfed at 1 month of age	100% (13/13)	100% (20/20	*NA*
Breastfeeding duration	7.7 (1.5 – 13.0)	10.5. (6.0 – 14.0)	0.031
Antibiotics before 6 months of age	15% (2/13)	5% (1/20)	0.32
Maternal prepregnancy BMI (mean, SD)	24.6 (18.4 – 33.6)	23.4 (19.7 – 38.5)	0.37
Maternal age (mean, SD)	35 (25 – 43)	34 (28 – 44)	0.31

Table 6. Clinical characteristics of the neonates exposed and non-exposed to neonatal antibiotics in Study I. Continuous data are expressed as means with range, and the differences between groups were assessed using Student's two-tailed t test. Dichotomous data are expressed as percentages (proportions) and were assessed using the Chi square test. NA = not applicable.

4.2.2 Methods

4.2.2.1 Intrapartum antibiotics (III)

Infant fecal samples were obtained at the ages of 1 and 6 months and stored at −80°C until DNA extraction. Microbial DNA Extraction, Sequencing, and Statistical Methods Microbial DNA was extracted from approximately 200 mg of stool using

an automated InviMag® Stool DNA Kit (Stratec Molecular, Berlin, Germany) with a KingFisher magnetic particle processor (Thermo Fisher Scientific Oy, Vantaa, Finland) with slight modifications (Hermansson et al., 2019). Primers targeting the V3-V4 region of 16S rRNA gene were used for amplification using 2 × 300-bp paired-end run Illumina MiSeq platform (FISABIO sequencing service, Valencia, Spain) (Klindworth et al., 2013). Sequence quality was assessed by PRINSEQ lite program (min_ length:50; trim_qual_right:20; trim_qual_type:mean; trim_qual_ window:20) (Schmieder & Edwards, 2011). QIIME (version 1.9.1, with default parameters), an open-source platform, was further used for sequence filtering, demultiplexing, and clustering to form operational taxonomic unit tables (97% identity) based on the Greengenes database. For the testing of the microbial differences among groups, operational taxonomic units present in <2 times in 10% of the samples were excluded. Samples with <1,000 reads were also excluded, resulting in removal of only one sample from the dataset. Diversity metrics including alpha diversity (Shannon-Wiener index and Chao1 index for richness) and beta diversity (Bray-Curtis index) were used to test significance. The impact determination of differentially abundant bacterial genera between infant gut was tested using Analysis of Compositions of Microbiomes with Bias Correction (ANCOM-BC) modeling with the default parameters, and taxa with presence <10% were excluded (H. Lin & Peddada, 2020). This test estimates a change between test groups for each taxon using log-transformed values of absolute sequence counts. p values <0.05 were regarded as statistically significant, and significance was assessed with a threshold of false discovery rate <0.05. Predictive inferred functional analysis was performed using PICRUSt pipeline on the online Galaxy interface (http://huttenhower.sph.harvard.edu/galaxy/). PICRUSt was used to derive relative Kyoto Encyclopedia of Genes and Genomes pathway abundance. The linear discriminant analysis effect size was performed for the biomarker discovery using a size-effect cutoff of 3.0 on the logarithmic linear discriminant analysis score (Segata et al., 2011).

4.2.2.2 Neonatal antibiotics (I)

Fecal samples from infants were collected at the ages of 1, 6, 12, and 24 months, and the fecal microbiota was analyzed by sequencing of the 16 S rRNA gene and metagenomics for a subset of the children. DNA was extracted from infant and stool samples using the Power Soil DNA Isolation Kit (MoBio) according to the manufacturer's instructions, using a Beadbeater (BioSpec) for 2 min. Following DNA extraction, the V4 variable region of the bacterial 16 S rRNA gene was amplified by polymerase chain reaction (PCR) using the 515 F and 806 R primers, and each sample received a unique 515 F barcoded primer, in order to identify each

sample during data analysis, as described in the **Study I**. PCR reactions were carried out with the Primestar taq polymerase (Takara) for 30 cycles of denaturation (95 °C), annealing (55 °C) and extension (72 °C), and a final elongation at 72 °C. Products were purified using AMPure magnetic beads (Beckman Coulter) and quantified using Pico-green dsDNA quantitation kit (Invitrogen). Samples were pooled at equal concentrations (50 ng/µl), loaded on 2% E-Gel (Thermo Fisher), and purified using NucleoSpin Gel and PCR Clean-up (Macherey-Nagel). Purified products were sequenced using the Illumina MiSeq platform (Genomic Center, Azrieli Faculty of Medicine, BIU, Israel). For the shotgun samples, libraries were prepared with the NexteraXT DNA Library Preparation Kit (Illumina) and sequenced on the HiSeq2500 and NovaSeq machines (Illumina). The 27 samples were sequenced for a total of 76.9 Gb (2.8 Gb average per sample after quality control, 1.1 Gb standard deviation). The raw reads were submitted to the NCBI-SRA archive and are available under BioProject PRJNA606271.

Given the large variation in feature values, these values were transformed to z-scores by adding a minimal value to each feature level (0.01) and calculating the log10 of each value. Statistical whitening was then performed on the table, by subtracting the average and dividing by the standard deviation of each feature. This process was then repeated for each patient.

4.3 Gut microbiota as a potential mediator (I)

4.3.1 Materials

In **Study I**, GF Swiss Webster mice were used for the animal experiment and maintained at the animal facility at the Azrieli Faculty of Medicine. GF mice that underwent fecal transplantation were housed in the conventional animal facility in regular cages and under standard housing protocols. All animals were housed under 12 h light–12 h dark regime and had free access to food and water; all mice were fed from the same food batch (Harlan-Teklad). The experiment was performed using protocols approved by the Bar Ilan University Animal Studies Committee.

4.3.2 Methods

Fecal samples from each infant (as described in 4.2.2.2) were transplanted to GF mice by oral gavage at 5 weeks of age. Each sample was suspended in 800 µl of sterile phosphate-buffered saline (PBS) and dissolved by vortex for 1-min. A total of 200 µl of the fecal suspension was administered by oral gavage to one to three GF mice. The process took place once, immediately after the mice were taken out of the isolator. To minimize cage effects, each treatment group was housed in at least two

cages, while mice transplanted with the same sample were not housed in the same cage. Mice were followed for 43 days, and day 0 refers to the day of the fecal transplantation. Stool microbiota and weight was examined at seven time points (days 0, 3,7,14,21,35,43). Stool samples were stored at −80 °C.

Mouse body weight was normalized according to day 0, the first day of the experiment, to calculate changes between Abx and control transplanted mouse groups over time. Differences in weight gain fold change were assessed using unpaired two-tailed t-test. All weight data are mean ± SEM. Unsupervised Learning was performed on the rarefied and subsampled version of the 16 S rRNA feature table to recognize patterns in the data. Principal Component and Principal Coordinates Analysis were performed using Python version 3.5, and its sklearn package. A 2-tailed p-value of less than 0.05 was taken to indicate statistical significance. Asterisks indicate significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$).

4.4 Ethical considerations

This research adheres to the ethical principles of the Declaration of Helsinki, with particular attention to the vulnerability of neonates and children. Avoiding unnecessary interventions and treatments was central to the study design. **Studies I and II**, based on register and cohort data, involved no contact with families and no interventions; accordingly, no informed consent was required. The nature of these studies posed neither risks nor direct benefits to the study population. Both studies were deemed ethically acceptable and approved by the Finnish Institute for Health and Welfare (THL), a national expert agency under the Ministry of Social Affairs and Health. In Study II, the processing of personal data was conducted under the legal basis of public interest and scientific research (EU General Data Protection Regulation 2016/679, Articles 6(1)(e) and 9(2)(j); Data Protection Act, Sections 4 and 6).

Study III was approved by the Ethics Committee of the Intermunicipal Hospital District of Southwest Finland and registered (NCT00167700); oral and written consent was obtained from the mothers in accordance with national and international standards, as the study included collection of stool samples.

More broadly, mechanistic aspects that cannot be ethically studied in children may be explored in animal models, provided that the principles of replacement, reduction, and refinement are observed, as done in **Study 1**. Across all studies, particular attention was paid to data protection. Finally, studies addressing socioeconomic disadvantage were reported in a way that avoids stigmatization and aims to ensure that research benefits reach the populations most affected.

5 Results

5.1 Early exposures and childhood growth

5.1.1 Neonatal antibiotic exposure and growth (I)

Altogether 9.3 % of the neonates in the **study I** (1,151/12,422) had been exposed to antibiotics within the first 14 days of life. A vast majority of the subjects were treated with a combination of intravenous benzylpenicillin and gentamicin as per the hospital guidelines. Antibiotic treatment was categorized to brief empirical antibiotic treatment, which was discontinued after infection had been ruled out (4.1% of the total study population, 513 neonates) and to antibiotics administered for confirmed or clinical infection (5.1% of the study population, 638 neonates) to differentiate between the impact of antibiotic exposure and the underlying infection. The background and clinical characteristics of the neonates are presented in detail in Table 2 in 4.1.2.1.

After adjusting for confounding factors as described in 4.4.1, boys exposed to brief empirical antibiotic exposure or antibiotic treatment for infection exhibited significantly lower weight compared to non-exposed children throughout the first six years of life ($p = 0.007$ for infected boys, and $p = 0.031$ for non-infected boys, Fig. 5a). From 2 to 6 years of age, boys who received antibiotics to treat infections were also significantly shorter ($p = 0.011$; Fig. 5c) and had a lower BMI ($p = 0.037$; Fig. 5e) than boys without antibiotic exposure. There was no statistically significant association between neonatal antibiotic exposure and weight, height, nor BMI (Fig 5b, d, f) during the first six years of life in girls after adjusting for the same potential confounders.

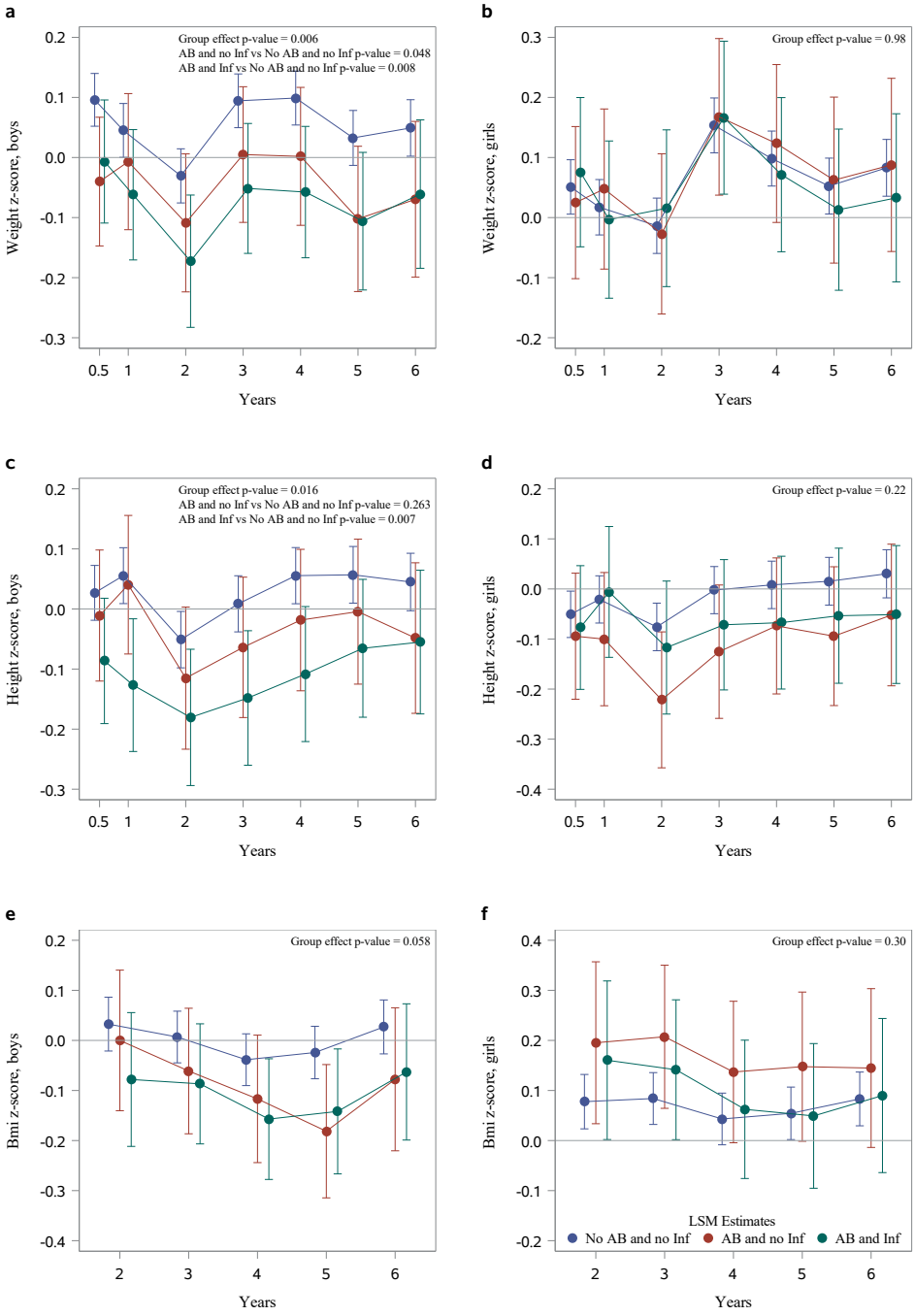


Figure 5. Childhood growth after antibiotic exposure with infection (green), antibiotic exposure without infection (red), and without antibiotic exposure nor infection (blue) in boys (a, c, e) and girls (b, d, f). The whiskers represent 95% confidence intervals. Please note the different scales in the Y-axes.

5.1.2 Childhood antibiotic use and growth (I)

The cumulative number of antibiotic courses after the neonatal period but during the first six years of life was associated with significantly higher weight z-scores during the first six years of life in boys (Fig. 6a, $p < 0.001$) but not in girls (Fig. 6b, $p = 0.71$). Height development during the first six years of life was not associated with antibiotic use after the neonatal period in either boys (Fig. 6c) or girls (Fig. 6d).

Higher BMI z-scores were significantly associated with the number of antibiotic purchases in both boys (Fig. 6e, $p<0.001$) and girls (Fig. 6f, $p < 0.001$). This was consistent throughout the study period in a hierarchical linear mixed model for repeated measurements adjusted for gestational age, birth weight z-score, mode of delivery, maternal prepregnancy BMI and neonatal antibiotic exposure.

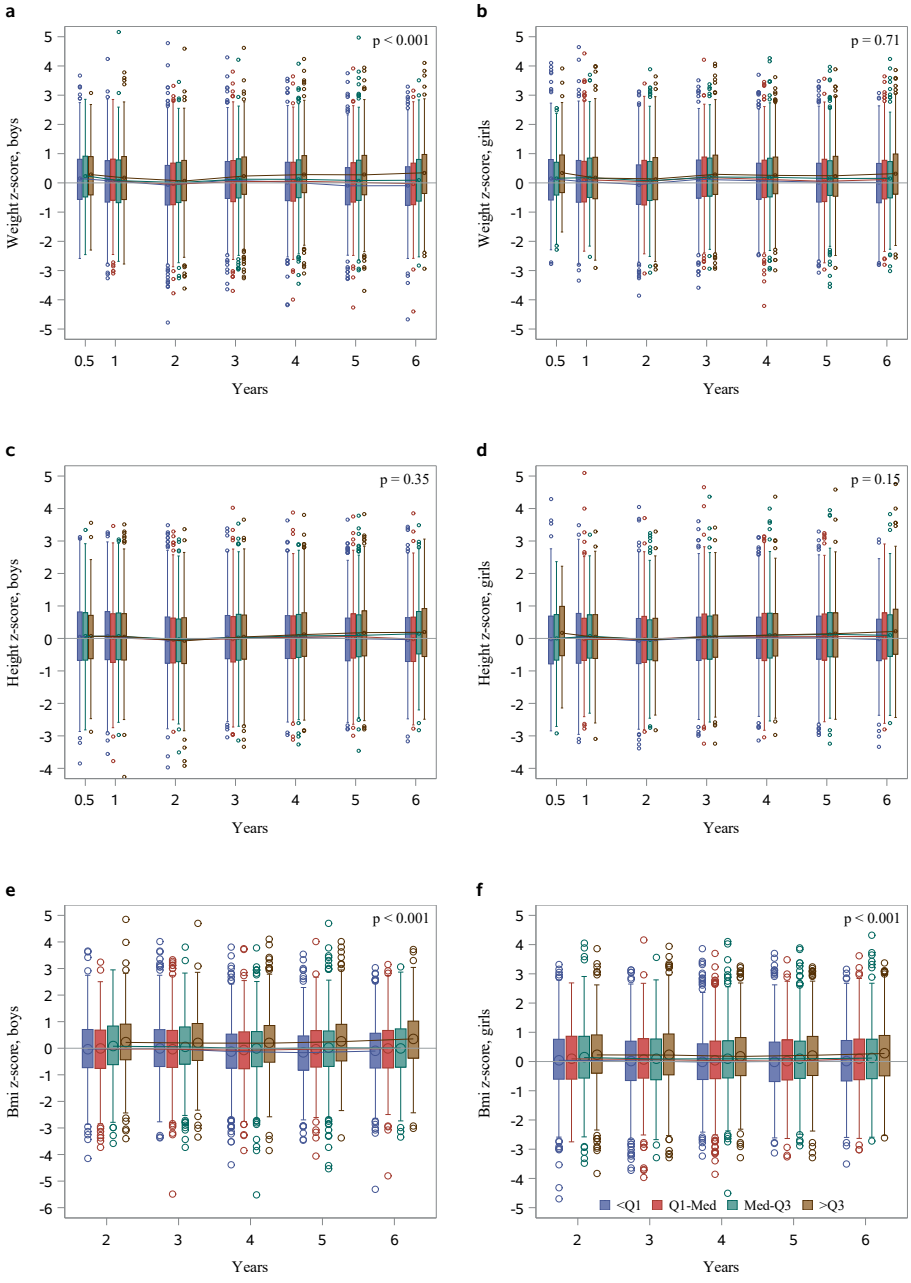


Figure 6. The association between early childhood cumulative antibiotic exposure and growth during the first six years of life in boys (a, c, e) and girls (b, d, f). The subjects have been categorized by quartiles (Q1, median, and Q3) based on the cumulative number of antibiotic purchases at each point in time. The boxes represent interquartile range (IQR), and the whiskers represent 1.5 times IQR. The circles represent outliers.

5.1.3 Neighborhood socioeconomic status and growth (II)

Cumulative neighborhood socioeconomic disadvantage was associated with recognizable childhood BMI z-score trajectories from birth to age 7. Trajectories began to diverge at 4 years of age, as children growing up in the most disadvantaged neighborhoods exhibited a trajectory of increasing BMI z-scores, despite being born in the lowest BMI z-scores (Figure 7).

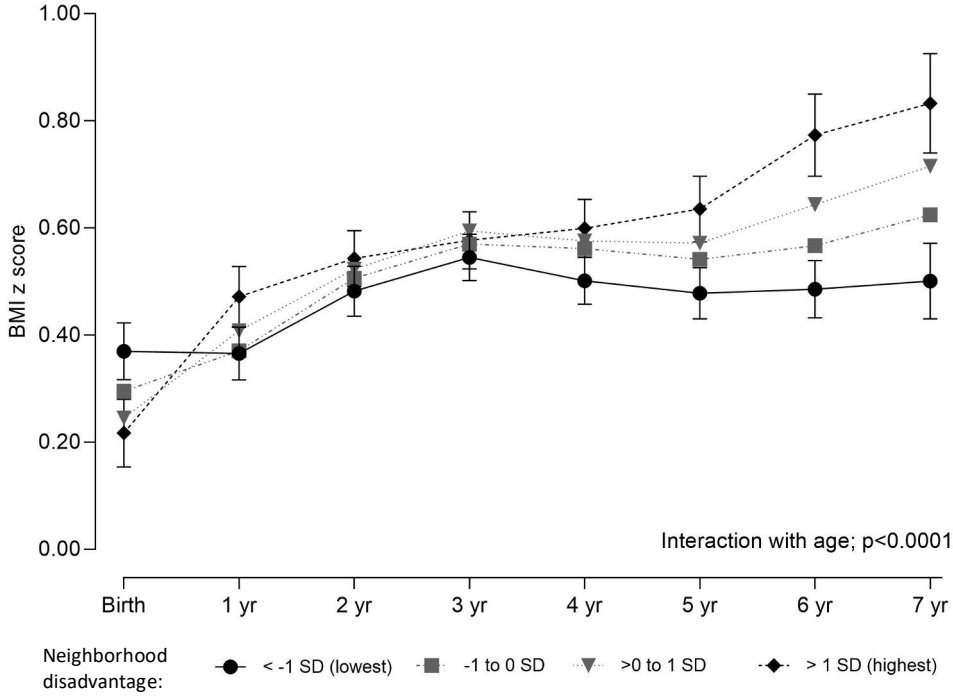


Figure 7. BMI z-score trajectories in children exposed to cumulative neighborhood socioeconomic disadvantage. BMI z-scores are expressed as mean values and their 95% confidence intervals from birth to 7 years of age. The marginal structural GEE models with inverse probability weighting are adjusted for child sex, preterm birth, maternal age, primiparity, single parenthood, immigrant background, smoking during pregnancy, prepregnancy obesity, gestational diabetes mellitus, other maternal medical conditions during pregnancy, and parental socioeconomic status. No interaction with child sex was detected (P = 0.54). Disadvantage categories are based on national standardized mean score.

Children growing up in disadvantaged neighborhoods also presented with a higher risk of overweight or obesity at the end of the follow-up as compared with children living in more affluent neighborhoods (30% vs 22% and 9% vs 5%, respectively) (Figure 8).

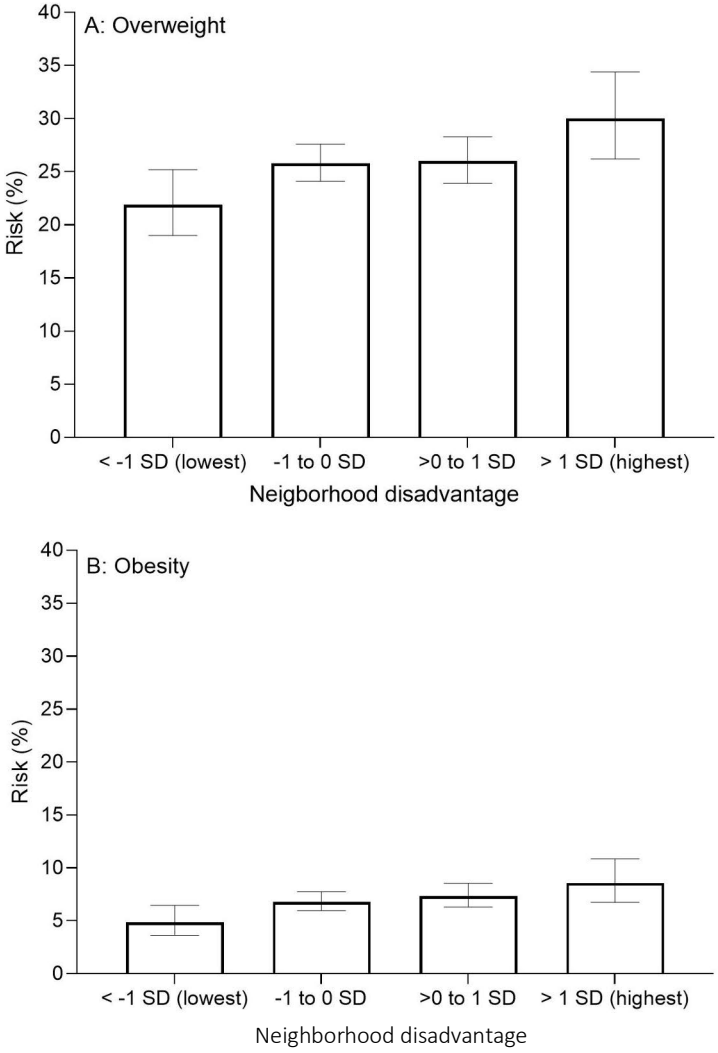


Figure 8. Cumulative neighborhood disadvantage and risk of (A) overweight and (B) obesity at age 6–7 years. Overweight is defined as BMI z-score > +1 SD and obesity as BMI z-score > +2 SD. Only those with a completed follow-up were included in the analysis (N = 8,021). The models were adjusted for child sex, preterm birth, maternal age, primiparity, single parenthood, immigrant background, smoking during pregnancy, prepregnancy obesity, gestational diabetes mellitus, other medical conditions during pregnancy, and parental socioeconomic status. The whiskers represent the 95% confidence interval. No interaction with child sex for overweight (P = 0.43) or obesity (P = 0.43) was detected.

5.2 Impact of antibiotic exposure on the gut microbiota

5.2.1 Intrapartum antibiotic exposure (III)

The clinical and background characteristics of the infants exposed and not exposed to IAT are presented in Table 7, and the groups were comparable. The most common reason for intrapartum antibiotic administration among the study subjects was maternal recto-vaginal colonization with group B Streptococcus (GBS), and consequently, all used antimicrobial agents were beta-lactams (benzylpenicillin or cephalosporins), of which benzylpenicillin was administered to 19/24 subjects. The association between infant sex and maternal probiotic administration to infant gut microbiota composition was analyzed: as differences were not detected, potential confounding was ruled out.

	IAT (n=24)	**no IAT (n=24)**	**P**
Gestational age (weeks)	39.8 (37.6 – 41.9)	39.6 (37.6 – 42.3)	0.61
Boys	17 (71%)	11 (46%)	0.08
Birth weight (grams)	3434 (2850 – 4100)	3458 (2430 – 4220)	0.85
Breastfed at 1 month of age	24 (100%)	24 (100%)	
Breastfed at 6 months of age	16 (66.7%)	18 (75%)	0.53
Maternal prepregnancy BMI (mean, SD)	24.6 (18.4 – 33.6)	23.4 (19.7 – 38.5)	0.37
Maternal age (mean, SD)	35 (25 – 43)	34 (28 – 44)	0.31
Antimicrobial treatment			
Benzylpenicillin iv	19/24		
*Cephalosporin iv**	3/24		
*Cephalosporin po***	2/24		
Indication for antimicrobial treatment			
Positive GBS screening	13/24		
Premature rupture of membranes	8/24		
*Maternal infection***	3/24		

* one mother was treated with cefuroxime for pneumonia; two mothers received cefalotin due to penicillin allergy
** one mother had UTI and one bronchitis, which both were treated with cephalexin; one mother had pneumonia treated with cefuroxime

Table 7. Clinical and background characteristics of the infants exposed and not exposed to intrapartum antibiotic treatment.

At 1 month of age, the gut microbiota richness was decreased in infants exposed to IAT as compared to subjects not exposed to IAT, while no difference in alpha diversity was detected (Fig. 9a, c). At the age of 6 months, the groups were comparable regarding both richness and diversity, as assessed by Chao1 index and Shannon index, respectively (Fig. 9b, d). The gut microbiota from subjects exposed and not exposed to IAT did not cluster separately in a statistically significant manner as assessed by the Bray-Curtis test at 1 (Fig. 9e) or 6 months of age (Fig. 9f).

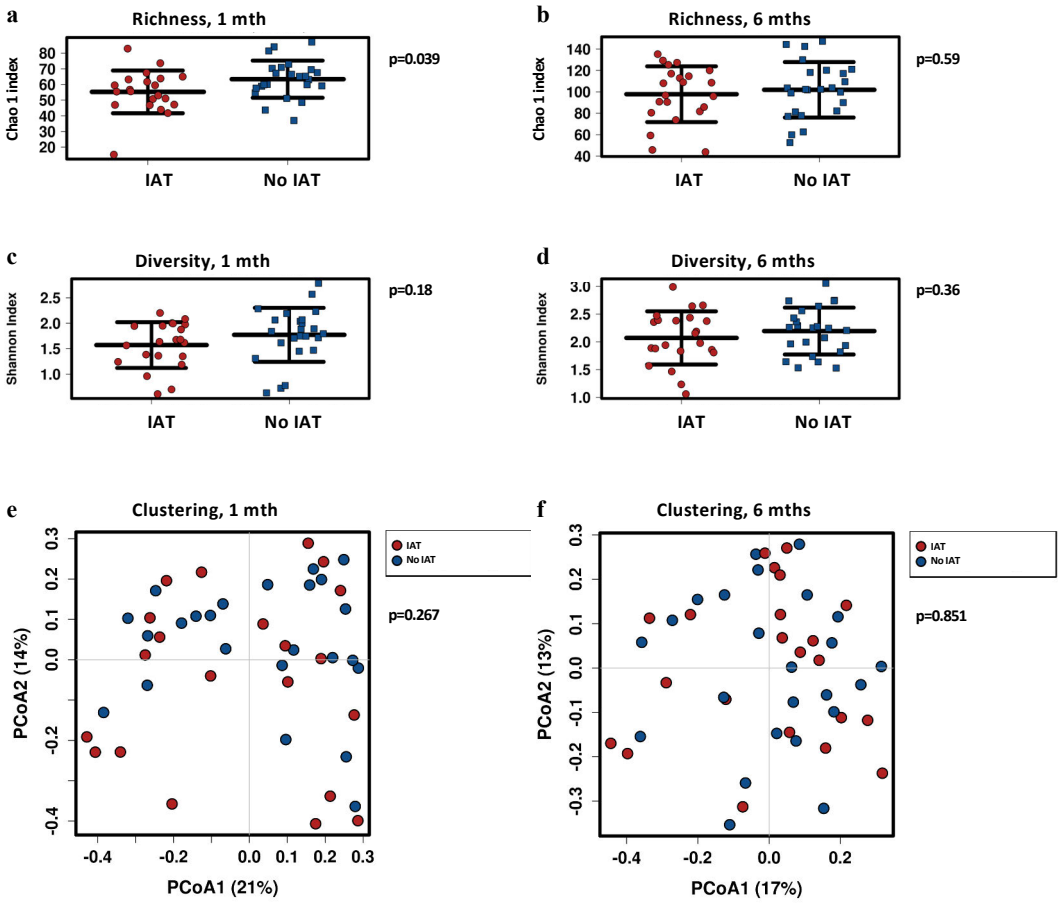


Figure 9. Gut microbiota richness, alpha and beta diversity in subjects exposed and not exposed to intrapartum antibiotic treatment.

Significant differences in the relative abundance of specific taxa were discovered at 1 month of age between subjects exposed and not exposed to IAT (Table 8).

a) 1 month

Phylum	Family	Genus	beta_coef	SE	W	p	p adj.	diff_abun
Firmicutes	*Erysipelotrichaceae*	*unclassifiedErysipelotrichaceae*	1,96	0,65	3,00	<0,05	<0,05	TRUE
TM7	*unclassifiedTM7*	*unclassifiedTM7*	0,095	0,29	0,33	<0,05	<0,05	TRUE
Firmicutes	*Clostridiaceae*	*Clostridium*	-1,08	0,52	-2,10	<0,05	<0,05	TRUE
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	1,03	0,52	1,99	<0,05	<0,05	TRUE
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	1,97	0,94	2,09	0,036	0,427	FALSE
Firmicutes	*Lachnospiraceae*	*Blautia*	-0,76	0,48	-1,57	0,115	0,819	FALSE

b) 6 months

Phylum	Family	Genus	beta_coef	SE	W	p	p adj.	diff_abun
Actinobacteria	*Propionibacteriaceae*	*Propionibacterium*	-0,05	0,29	-0,17	<0,05	<0,05	TRUE
Actinobacteria	*Coriobacteriaceae*	*Collinsella*	1,02	0,73	1,40	<0,05	<0,05	TRUE
TM7	*unclassifiedTM7*	*unclassifiedTM7*	0,62	0,27	2,30	0,022	0,938	FALSE
Firmicutes	*Enterococcaceae*	*unclassifiedEnterococcaceae*	-0,66	0,40	-1,67	0,095	0,938	FALSE
Actinobacteria	*Bifidobacteriaceae*	*Bifidobacterium*	0,80	0,78	1,03	0,30	0,938	FALSE

Table 8. Results from the ANCOM-BC log-linear model to determine relative abundancies of specific taxa in infants exposed and not exposed to intrapartum antibiotic treatment at 1 month (a) and 6 months (b) of age. In the table: beta_coef = coefficients (beta-coefficient); SE = standard errors; W = test statistics; p = p-values; p adj. = adjusted p-values; diff_abun = indicators whether the taxon is differentially abundant (TRUE) or not (FALSE).

There was a statistically significant increase in the genus *Clostridium* in the fecal microbiota of IAT exposed infants. Furthermore, a significant reduction was seen at family level in *Erysipelotrichaceae* and the genus *Collinsella* (Fig. 10a). At the age of six months, the relative abundance of *Collinsella* remained lower in infants exposed to IAT, and the relative abundance of the genus *Propionibacterium* was also lower. No statistically significant differences in the relative abundance of other specific taxa were detected to extend to the age of 6 months (Fig. 10b).

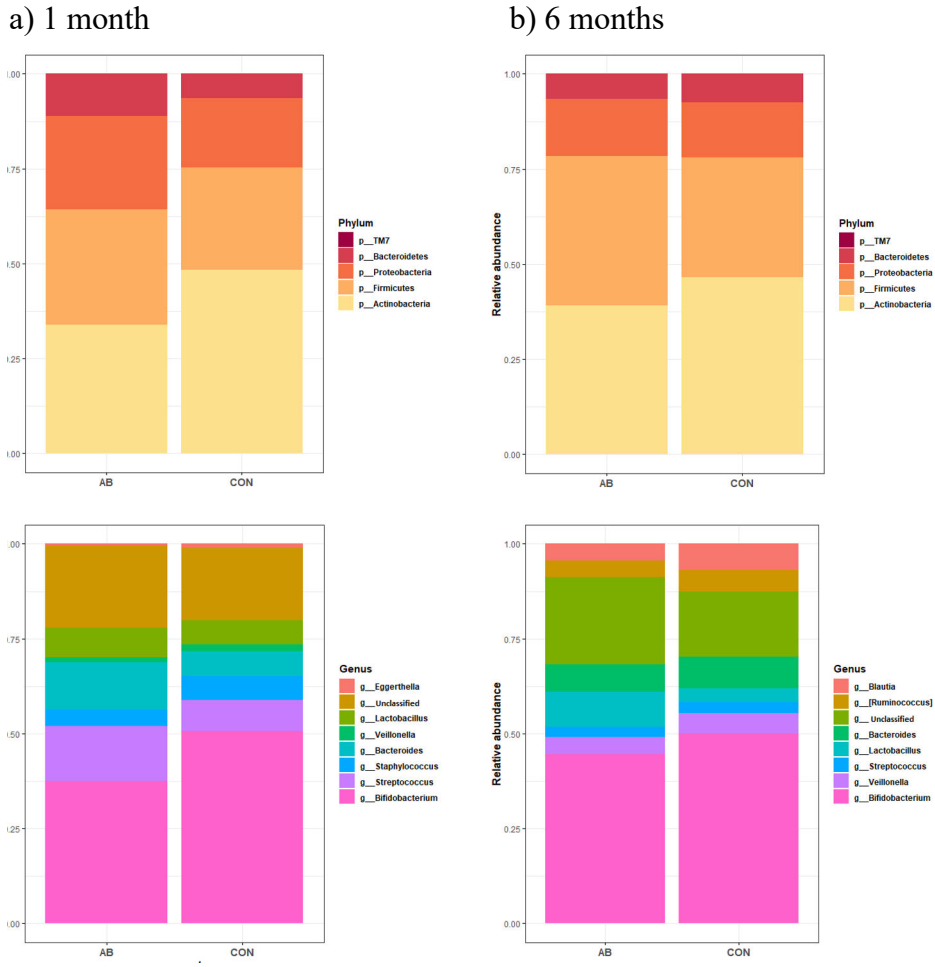


Figure 10. Relative abundancies of specific taxa at 1 month (a) and 6 months (b) of age.

5.2.2 Neonatal antibiotic exposure (I)

Neonatal antibiotic exposure was associated with significant alterations in the gut microbiota throughout the study period. Weighted and Unweighted UniFrac analysis showed a gradual change between 1–24 months in bacterial composition, reflecting the maturation of the gut microbiota in the children not exposed to neonatal antibiotics (Fig. 11a, c, respectively). The subjects exposed to neonatal antibiotics also demonstrated an expansion of bacterial establishment over time but in a distinct and less organized pattern (Fig. 11b, d).

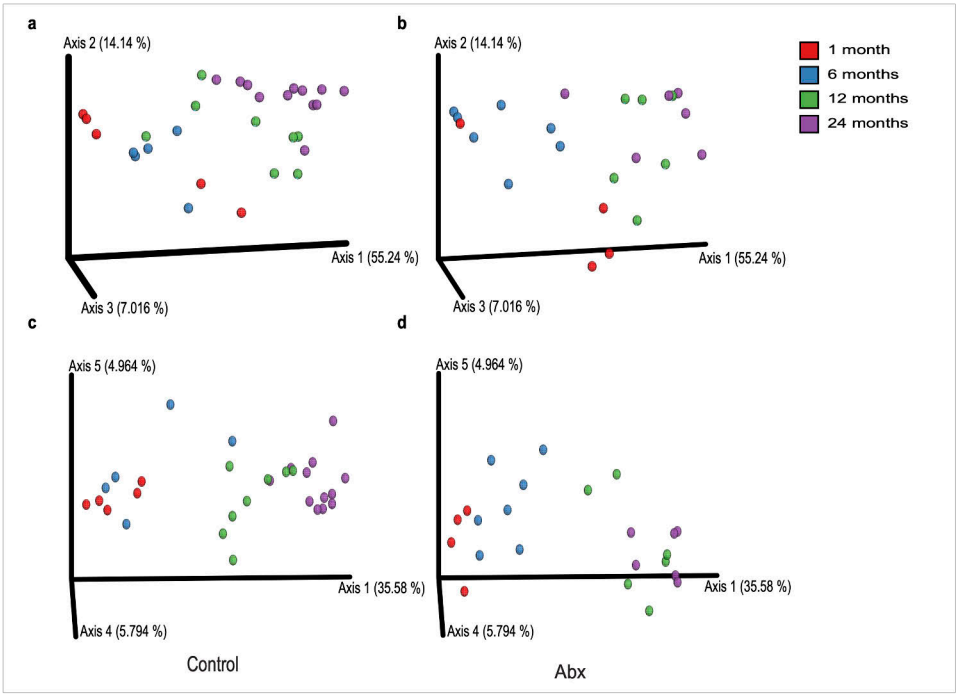


Figure 11. Alterations in gut bacterial colonization in infants following antibiotic exposure presented in PCoA based on Weighted UniFrac (a, b) and Unweighted UniFrac (c, d) distance matrices. Controls (a, c; n = 20), and antibiotic-exposed (b, d; n = 13) infants after 1 (red), 6 (blue), 12 (green), and 24 (purple) months from antibiotic exposure.

The infants exposed to neonatal antibiotics exhibited significantly lower gut microbiota richness as compared to the non-exposed infants at the age of 1 month ($p = 0.014$) (Fig 12a). Interestingly, at the age of 6 months, the antibiotic-treated infants reached the bacterial richness level of the control infants, and at the ages of 12 and 24 months, the antibiotic-treated subjects gained significantly higher levels of bacterial richness as compared to the control subjects ($p = 0.014$ and $p = 0.001$, respectively). (Fig. 12a).

Taxonomic analysis based on genus-level relative abundancies identified *Bifidobacterium* as the genus most notably affected by neonatal antibiotic treatment, with the effect persisting up to 24 months of age (Fig. 12b-d). Bifidobacteria accounted for approximately 50% of total sequences at 1 and 6 months of age in infants not exposed to antibiotics, declining to ~25–30% by 12 and 24 months (Fig. 12b-d). In contrast, antibiotic-exposed infants showed consistently lower Bifidobacterium abundance across all time points – except at 6 months post-treatment – compared to controls.

Feature-level analysis revealed that the *Bifidobacterium* genus consisted of five unclassified features. Antibiotic-treated infants exhibited lower diversity in fecal *Bifidobacterium* features than controls at each time point. Notably, the temporary increase in Bifidobacterium abundance at 6 months in the treated group was predominantly driven by a single feature (unclassified1). Meanwhile, features unclassified2 and unclassified3 were found primarily in control infants (Fig. 12c). Differences in other bacterial genera between the antibiotic-treated and control groups across time points are summarized in Fig. 12d.

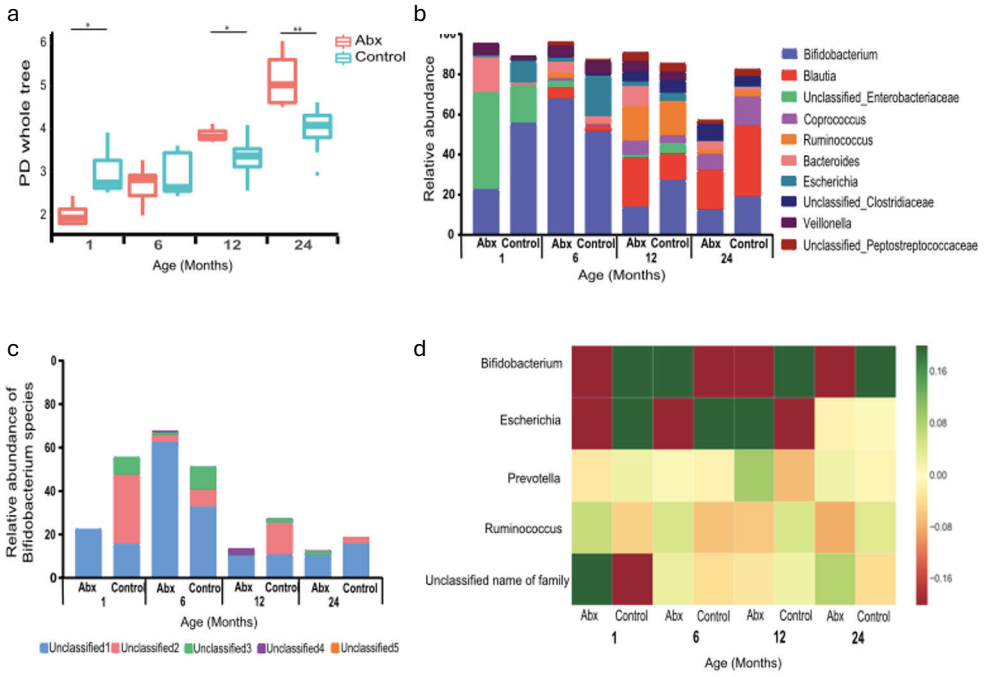


Figure 12. Alpha diversity comparison based on phylogenetic diversity (a); Relative taxonomic composition based on 10 most abundant genera of control and antibiotic-treated groups (b); Relative levels of Bifidobacterium species (c); Average log10 change in the bacteria showing significant differences between groups ($p < 0.05$ and FDR correction) (d). Green values imply over-representation, and red represents under representation. (*$p < 0.05$, **$p < 0.01$; minimum four samples in each group).

5.3 Gut microbiota as a potential mediator

Male mice that received fecal microbiota transplants (FMT) from infants one month after antibiotic exposure showed a significantly reduced relative weight gain over the 43-day follow-up, compared to those receiving FMT from unexposed infants. This difference became statistically significant from day 7 post-transplantation ($p \leq 0.001$; Fig. 13a). In contrast, female mice given FMT from antibiotic-exposed infants exhibited only a temporary reduction in growth, observed at day 3 post-transplant ($p = 0.02$; Fig. 13b).

Growth impairment was observed in male mice also when FMT was performed with samples collected 24 months after antibiotic exposure (Fig. 14a).

FMT from 1-month-old infants not exposed to antibiotics led to a sustained increase in bacterial richness from day 3 post-transplantation until the end of the experiment (Fig. 13c). In contrast, mice receiving FMT from infants one month after neonatal antibiotic exposure showed an increase in richness only between days 3 and 7, after which levels stabilized. As a result, significant differences in bacterial richness between the groups were observed at four time points: days 14, 21, 35, and 43 (Fig. 13c). No such differences were found in mice receiving FMT from 24-month-old infants (Fig 14b).

Differences in bacterial community composition (beta diversity) were assessed across seven time points in mice receiving FMT from antibiotic-exposed versus control infants. Mice given FMT from antibiotic-treated infants displayed distinct temporal clustering of samples from day 3 to day 43, compared to those receiving FMT from age-matched controls. (Fig. 13d, e; Fig. 14c, d).

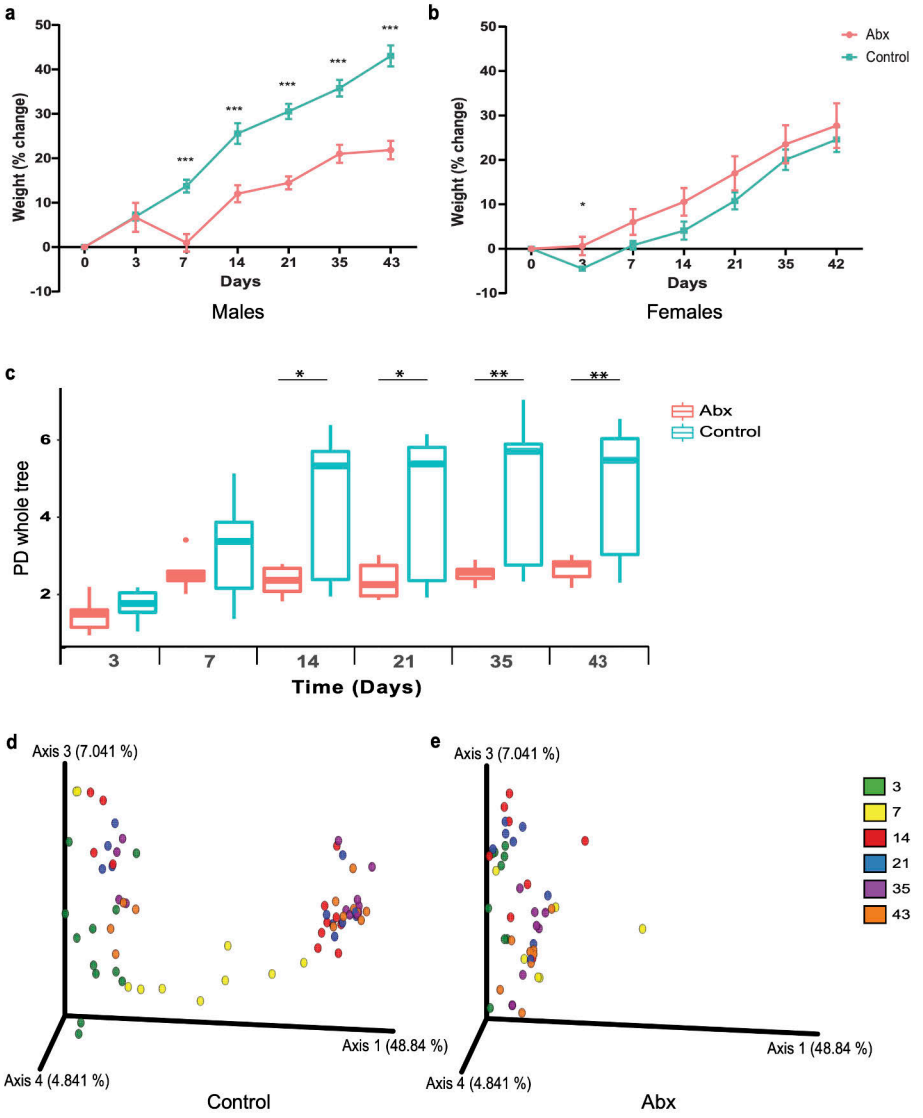


Figure 13. a) Male mice receiving FMT from 1-month old infants exposed to neonatal antibiotics (pink) gained significantly less weight compared to mice receiving FMT from non-exposed infants (blue) starting from day 7 (***p ≤ 0.001); b) Female mice receiving FMT from antibiotic-exposed neonates (pink) exhibit only a transient difference in growth 3 days after transplantation (p = 0.02). c) Fecal microbiota alpha diversity comparison based on phylogenetic diversity; PCoA based on Unweighted UniFrac distances in mice receiving FMT from control (d) antibiotic-exposed (e) infants at six-time points following FMT. (*p < 0.05, **p < 0.01, ***p < 0.001 and ****p < 0.0001; weight data represent the mean ± SEM of at least four samples in each group).

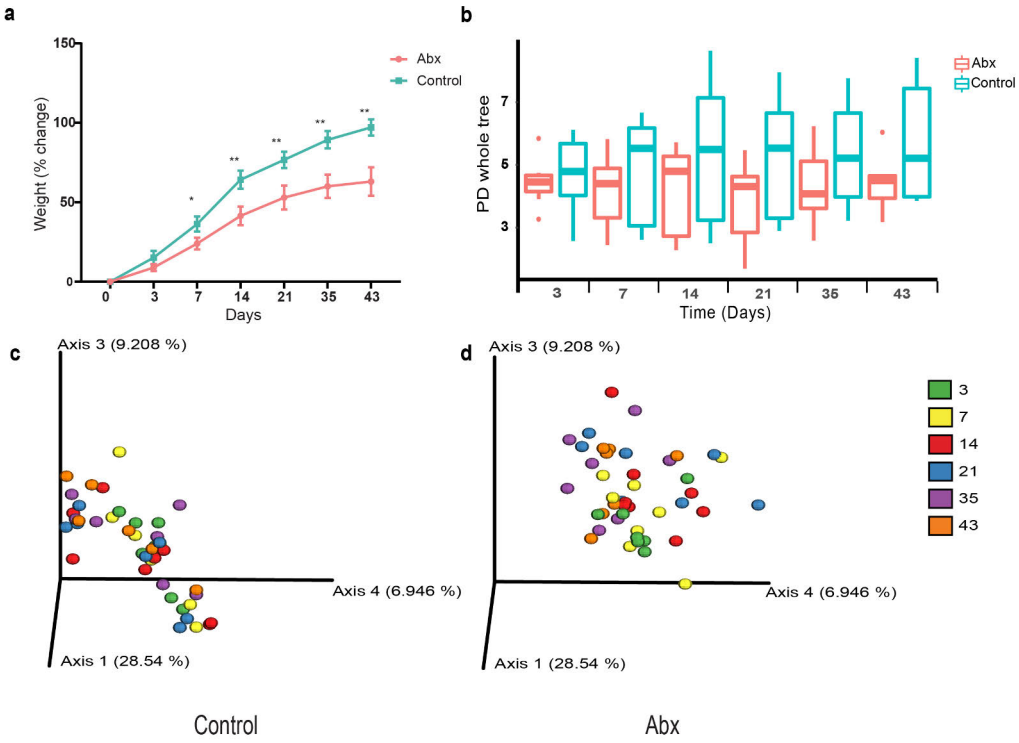


Figure 14. FMT from antibiotic-treated infants, 24 months after exposure, to GF mice induces changes in weight gain and bacterial composition. (A) Mice receiving fecal transplantation from antibiotic treated infants (pink) gained significantly less weight (expressed as mean +/- SEM) compared to control animals (blue) starting from day 7. (B) No significant changes were seen in alpha diversity comparison based on phylogenetic diversity. (C) PCoA based on Unweighted UniFrac distance matrix in control, and (D) Abx mouse groups at six time points following FMT. (*p < 0.05, **p < 0.01).

6 Discussion

In this study I found that early childhood factors, such as neonatal antibiotic exposure and neighborhood socioeconomical status, have long-lasting effects that are visible in childhood growth. Even more importantly, gut microbiota appears as a potential mediator.

6.1 Growth and exposures

6.1.1 Antibiotic exposure

Early neonatal antibiotic exposure correlates with growth disturbances, as demonstrated in **Study I**. Exposure to antibiotics during the immediate neonatal period was associated with a downward shift in the growth curve. In boys, weight and height gain were significantly attenuated during the first six years of life, while no such effect was observed in girls after adjusting for potential confounders.

These results highlight three key points: first, antibiotic exposure influences growth; second, the duration and intensity of exposure matter; and third, the effect differs between sexes.

The association of neonatal antibiotic exposure and childhood growth is studied surprisingly poorly, and most of the small studies comprise preterm babies. Furthermore, results from these are conflicting. Consistently with our results, a recent study by Lin et al (Y. C. Lin et al., 2024) evaluated growth trajectories of 481 preterm neonates following antibiotic exposure, and concluded that antibiotic exposure duration was negatively associated with growth measurements up to 5 years of age.

Pyle et al received conflicting results, although they studied premature very-low-birth-weight infants with a hypothesis of a positive association between antibiotic exposure and growth. In their retrospective study antibiotic therapy was not associated with weight or length delta z-scores from birth through 12 months corrected age. (Pyle et al., 2021) Similar results were seen in a study by Reid et al, as antibiotic exposure to preterm infants (born 30-32$^{6/7}$) was not associated with growth velocity changes until discharge. (Reid et al., 2019) However, these studies

examining the impact of antibiotic exposure on the growth of preterm infants are only partially generalizable. Compared to full-term infants, preterm infants are relatively frequently exposed to antibiotics. Moreover, growth disturbances present at birth or during the neonatal period are common, and there are numerous confounding factors.

The age at which the antibiotic exposure occurs may have an important impact on later growth. In the present study, neonatal antibiotic exposure was linked to reduced weight and height gain in boys. In contrast, antibiotic use later in infancy and childhood was associated with increased weight in boys and higher BMI in both boys and girls during the first six years of life. These findings suggest that antibiotic exposure during the neonatal period may be causally related to reduced growth but antibiotic use later in infancy and childhood may have the opposite effect and increase the risk of obesity or becoming overweight. Importantly, the growth impairment appeared to be somewhat more pronounced in neonates who received a full course of antibiotics compared to those who were exposed to short empirical treatment. This finding could indicate a potential dose–response relationship.

Our results align with prior studies showing that antibiotic use after the neonatal period correlates with increased BMI. Aris et al., in a cohort study of over 183,000 children in the US, found that antibiotic exposure altered BMI trajectories (Aris et al., 2021). Similarly, in a Finnish cohort Saari et al. demonstrated that antibiotic exposure in infancy was most strongly linked to overweight status when it occurred before six months of age or was repeated frequently (Saari et al., 2015). Bailey et al. reported that repeated broad-spectrum antibiotic exposure before 24 months of age was associated with early childhood obesity in a cohort of 64,580 children (Bailey et al., 2014).

Additional large-scale studies further support these findings. In a large cohort with register data from the US (Block et al., 2018) a small association between antibiotic use before 24 months of age and higher BMI z-scores and overweight or obesity prevalence at 48 to 72 months of age was noted, including a modest dose response. Mbakwa et al. (2016) analyzed 979 children in the Netherlands and concluded that repeated early-life antibiotic exposure, particularly with beta-lactam antibiotics, was associated with increased weight and height by age 10 (Mbakwa et al., 2016).

Despite strong evidence linking early-life antibiotic exposure to growth deviations, some conflicting studies exist. In a study by Rogawski et al (Rogawski et al., 2015) antibiotic use in the first 6 months was not associated with growth until the age of 3 years. However, the study population consisted of only 497 children from India, and possible malnutrition and recurrent illnesses likely complicate the assessment of these results. Interestingly, the same group investigated early-life antibiotic exposure in a larger multinational cohort from South America, South Asia

and Sub-Saharan Africa, and found out that antibiotic use in the first 6 months of age was associated with increased weight from 6 months to 2 years. (Rogawski et al., 2017)

Our findings reinforce the idea that antibiotic exposure in infancy may contribute to childhood obesity. However, it is difficult to disentangle potential effects of antibiotics from those of the infections prompting their use, or from other unmeasured factors. A randomized controlled trial (RCT) to eliminate confounding by indication is neither ethical nor feasible. While **Study I** effectively accounted for confounders in neonatal antibiotic use, later antibiotic treatments were assessed based on purchase data without considering underlying infections. Li et al. investigated this issue in a birth cohort of 312,702 infants and found that infection, rather than antibiotic use, was associated with childhood obesity risk. Their study categorized infants into three groups: no infection/no antibiotics, infection/no antibiotics, and infection/antibiotics, revealing also that 53% of the population had been exposed to antibiotics within the first 12 months. (D. K. Li et al., 2017)

As both early neonatal and later childhood antibiotic exposure appear to have a detectable clinical effect on growth, the use of antimicrobial agents must be judicious. (Klingenberg et al., 2020; Magalhães et al., 2021; Stocker et al., 2023) Bearing this in mind, antibiotic exposure incidence in our cohort must be critically evaluated. In the **Study I,** altogether 9.3% of newborn (1151/12422) were exposed to antibiotics in the early neonatal period, of which more than half (638/1151) were deemed to have a clinical infection requiring more than short empirical treatment. It is notable, that the percentage of antibiotic-exposed neonates is somewhat larger than in more recent reports. In a multicenter study from Europe, North America and Australia, 2.86% of more than 750,000 late-preterm and term babies received intravenous antibiotics during the first postnatal week. (Giannoni et al., 2022) However, the incidence ranged from 1.18 to 12.45% among networks. Data from The Netherlands reveals that in 4.6% of live-born neonates antibiotic therapy was started, and in 2.3% it was continued beyond 48 hours. (van Veen et al., 2024) Interestingly, their data also suggested a marked fluctuation in the incidence of antibiotic use between hospitals.

In Nordic countries the incidence of antibiotic exposure is more invariable. Gyllensvärd et al reported that 1.88% of all term and late-preterm babies born 2012-2020 in Sweden received antibiotics during the first week of life (Gyllensvärd et al., 2024). In Norway 2.6% of all term and late-preterm babies born in 2015-2019 received intravenous antibiotics during the first 28 days after birth (Mundal et al., 2021). In Finland 3.8% of all newborns were exposed to antibiotic treatment in 2023 (THL, 2024). It is notable, that 2.6% of all Finnish neonates received antibiotics longer than 2 days, and only 1.2% were exposed only to short course of empiric

antibiotics. However, the incidence of antibiotic exposure in neonates is slightly larger in university hospitals, partly due to centralization of preterm and high-risk pregnancies. Despite this, the incidence was significantly larger in our center (8.8% of all born children)(personal notice, THL, 2024), which fortunately is less than in the cohort population of this study.

It is notable that observational studies are prone to confounding by indication also regarding the main aim of this study: could the observed association between antibiotic exposure and growth deviation actually be driven by the underlying indication for the antibiotic treatment, rather than the antibiotic itself? The clinical signs that trigger empirical antibiotics also relate to later growth both directly and through plausible mediators such as microbiota, immune response, and nutrient absorption, creating back-door paths from indication to outcome (Fig. 15). The majority of infections in infancy are viral, and antibiotic use is primarily aimed at bacterial infections. Yet confirming a bacterial origin is often difficult. While viral infections are less common in neonates, signs and symptoms mimicking infection may reflect transitional physiology rather than true infection. Neonates exposed to antibiotic therapy provide an opportunity to investigate the impact of antibiotic exposure with little risk of reverse causation. However, confounding by indication may persist. It must be noted that the observed dose–response effect of antibiotic exposure may also be affected by the underlying illness – potentially part of the pathway to growth disturbances – so residual confounding cannot be fully excluded even after multivariable adjustment. However, in our cohort the proportion of culture-positive bacterial infections, and the thresholds for both initiation of empiric therapy and extension to a full course, were low (Räty et al., 2024).

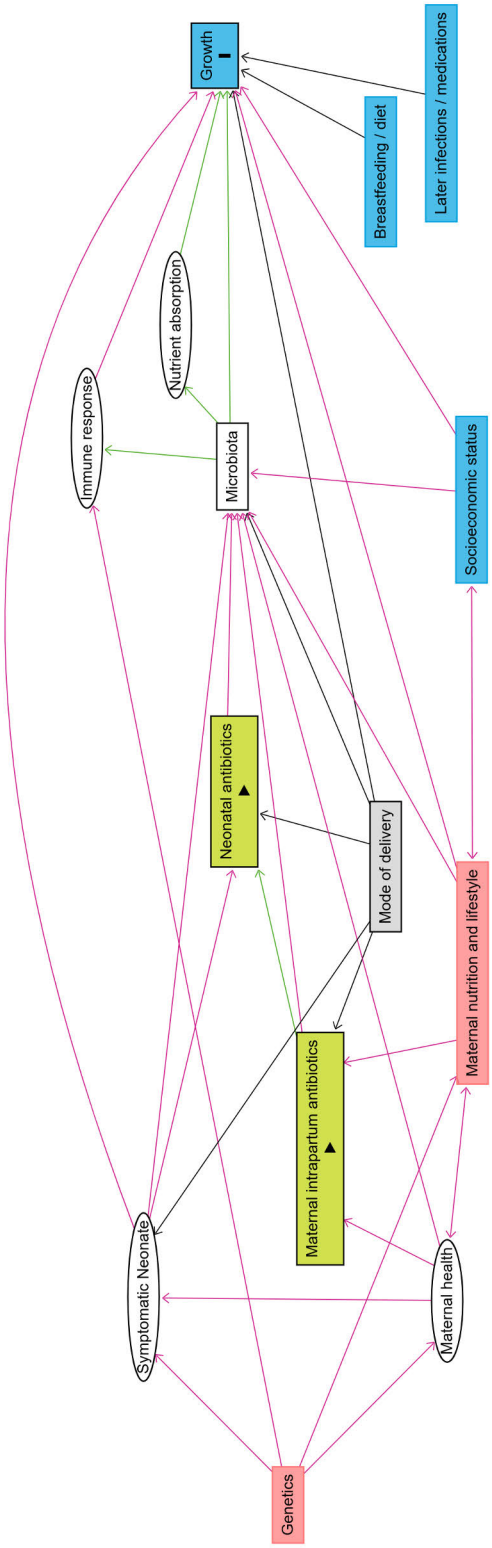


Figure 15. Exposures, outcome, and key confounding and background factors illustrated in the directed acyclic graph model.

6.1.2 Neighborhood socioeconomic status

In **Study II**, an association between cumulative neighborhood socioeconomic disadvantage and childhood BMI trajectories from birth to school age was evaluated. Children born in the most disadvantaged neighborhoods had the lowest BMI z-scores at birth but experienced an increase during their first year of life, a pattern not observed in those from the most affluent neighborhoods. Between ages 1 and 4, both groups showed a modest rise in BMI z-scores. However, after age 4, a clear divergence in trajectories emerged. Children exposed to the highest cumulative neighborhood disadvantage exhibited a continued increase in BMI z-scores, while those with the least exposure showed no change.

These findings align with data from the United States, where African-American children are born with lower birth weights than White children but follow steeper BMI growth trajectories (Isong et al., 2018). One potential explanation for this pattern is the clustering of multiple risk factors associated with neighborhood disadvantage. In the present study, potential contributing factors such as preterm birth, primiparity, single parenthood, immigrant background, smoking during pregnancy, pre-pregnancy obesity, gestational diabetes mellitus, and other medical conditions during pregnancy were considered. However, the difference in BMI trajectories between children from disadvantaged and advantaged neighborhoods could not be attributed to these factors alone, suggesting the influence of a broader range of environmental and social determinants.

Previous research on the association between neighborhood socioeconomic status and health has primarily focused on adults. Among middle-aged populations, cumulative neighborhood socioeconomic disadvantage has been linked to increased cardiometabolic risk factors and a higher incidence of diabetes mellitus and major cardiovascular diseases (Halonen et al., 2015; Kivimäki et al., 2018; Lagström et al., 2019; Ludwig et al., 2011; White et al., 2016) There are few studies suggesting similar associations between childhood neighborhood disadvantage and BMI or the risk of overweight or obesity in school age (Greves Grow et al., 2010), adolescence (Alvarado, 2016) and early adulthood (Lippert, 2016). Our current data provide comparable findings in Finland, a country with relatively small socioeconomic disparities, reinforcing the argument that neighborhood effects on health persist even in societies with strong social welfare systems. It is notable, that although family socioeconomic status is closely associated with neighborhood socioeconomic status, higher neighbourhood socioeconomic status has been shown to be more often associated with lower childhood obesity risk also after accounting for family-level socioeconomic status (Y. Kim et al., 2019).

Children with low birthweight often exhibit rapid postnatal growth, which is reportedly associated with increased risk of obesity (Woo Baidal et al., 2016). Notably, the accelerated increase in BMI z-scores leading to overweight and obesity

in our study began as late as age 4. This suggests that rather than being driven by prenatal influences, our results are consistent with the hypothesis that exposure to neighborhood socioeconomic disadvantage constitutes an important risk factor for the development of childhood obesity.

While living in a socioeconomically disadvantaged neighborhood is associated with an increased risk of obesity and overweight, evidence suggests that relocating to a more advantaged area may mitigate this risk (Ludwig et al., 2011). However, our findings indicate that children exposed to high neighborhood disadvantage continue to exhibit unfavorable BMI development even after adjusting for key confounders. Local environment and neighborhood socioeconomic status have previously been reported to be associated with maternal breastfeeding behavior, which may in turn modulate the risk of childhood obesity (Almquist-Tangen et al., 2013) Furthermore, neighborhood characteristics—including access to physical activity facilities, playgrounds, parks, food retail establishments, walkability, and perceived neighborhood safety—may mediate the associations observed in our study. While some research supports these factors as influential in childhood obesity risk, findings remain inconsistent across different populations and settings. Interestingly, in a recent Finnish report it was stated that the percentage of eight-graders with decreased physical functional capabilities was significantly higher in those living in neighborhoods with low socioeconomic status (Määttä & Sulander, 2025).

The Environmental Influences on Child Health Outcomes (ECHO) cohort has contributed valuable insights into the role of neighborhood environments in shaping child health. In a recent study by Aris et al. (Aris JAMA 2024) combined cohorts to examine associations of neighborhood food access and child BMI and obesity risk, and concluded that low-income, low-food access neighborhood residency posed an increased risk to higher childhood BMI and risk of overweight and obesity. From the same cohort residing in higher-opportunity and lower-vulnerability neighborhood in early life was associated with a lower risk of obesity from childhood to adolescence. (Aris et al., 2022) This finding is consistent with ours: children growing up in the most disadvantaged neighborhoods exhibited increasing BMI z-scores particularly after 4 years of age and a high prevalence of obesity at 7 years of age. These findings underscore the intergenerational nature of neighborhood disadvantage and its impact on childhood obesity risk.

Public planning and investment in neighborhood development play a crucial role in mitigating these disparities. Policies aimed at improving local environments, such as increasing access to nutritious food, enhancing opportunities for physical activity, and addressing broader socioeconomic inequalities, are essential in efforts to prevent childhood obesity and promote long-term health equity.

6.2 Gut microbiota disturbances

Antibiotic exposure causes changes in the gut microbiota, and the observed changes are dependent on the route, dose, and age of the individual. In **Study III,** intrapartum antibiotic treatment (IAT) given to the mother caused a transient change in the gut microbiota. More specifically, results indicate that IAT is associated with a reduction in overall neonatal gut microbiota richness and changes in relative abundances of specific taxa at 1 month of age. In **Study I**, neonatal antibiotic exposure resulted in a significantly altered gut microbiota which was characterized by disorganized development, increasing richness, and particularly reduced abundance and diversity of *Bifidobacterium* species.

The finding of **IAT affecting infant gut microbiota** extends previous reports based on quantitative PCR (Aloisio et al., 2014) or 16S rRNA gene sequencing (Aloisio et al., 2016) at 6–7 days of age. More specifically the phyla *Firmicutes* and *Actinobacteria* and particularly their families *Erysipelotrichaceae* and *Coriobacteriaceae* were found to be sensitive to IAT. The altered gut microbiota composition after IAT was also reflected in the gut microbiota function as assessed by predicted inferred functional analysis and the differences between exposed and nonexposed infants persisted in some degree to the age of 6 months.

The duration of the microbiota deviation associated with IAT exposure has remained an open question, although understanding of the effect of IAT on changes in diversity and differences at phylum, family, and genus levels, has increased. In a study by Corvaglia et al. (Corvaglia et al., 2016) infants of GBS-positive mothers exposed to IAT exhibited a decrease in bifidobacteria at 1 week of age. At the age of 30 days, the difference in the count of *Bifidobacterium* was no longer detectable. While a transient effect of IAT on *Bifidobacterium* was noted, the same effect was not seen in *Lactobacillus* or *Bacteroides fragilis*. Different analyzing methods may hinder the interpretation and repeatability. The study by Corvaglia consisted of 84 subjects in which fecal bifidobacteria were assessed by qPCR, which is a method that measures the number of copies of a DNA region defined by PCR primers and aims to measure quantity of specific microbe. Another method widely used is 16S rRNA gene sequencing, which was also used in the **Studies I** and **III**. It identifies a bacteria-specific RNA gene sequence and aims to analyze the microbial community more widely.

Stearns et al. analyzed fecal samples of infants born by CS (with antibiotic exposure) and vaginally with and without antibiotic exposure using 16S rRNA gene sequencing and reported that the impact of IAT administered for GBS prophylaxis on infant gut microbiota may persist beyond 12 weeks of age, albeit the most significant microbiota perturbations were observed during the first 6 weeks after delivery. IAT exposure in vaginally born infants was associated with gut microbiota alterations, specifically delayed colonization of Actinobacteria at 10 days and 6

weeks of age but no longer at 12 weeks of age. (Stearns et al., 2017) Additionally, a prospective cohort study of 198 healthy term infants from Canada reported that IAT was associated with gut microbiota disruptions, including reduced richness and underrepresentation of the genera *Bacteroides* and *Parabacteroides* at 3 months of age, but not at 12 months (Aloisio et al., 2014).

Zimmermann and Curtis have recently concluded in their systematic review that infants exposed to intrapartum antibiotics tend to have a lower relative abundance of Actinobacteria and reduced bacterial diversity until the follow-up age of 3 months. (Zimmermann & Curtis, 2019) In **Study III**, the decreased richness of the gut microbiota observed at 1 month of age was no longer detectable at 6 months of age. In addition, the observed changes in the relative abundances of specific taxa diminished over time. These findings suggest that IAT leads to a temporary disruption in gut microbiota colonization, which resolves within the first 6 months of life in healthy, term, and breastfed infants.

In **Study I**, exposure to antibiotics in the first days of life resulted in a significantly altered gut microbiota characterized by disorganized development, increasing richness, and particularly reduced abundance and diversity of *Bifidobacterium* species. More specifically, analysis showed a gradual change between 1–24 months in bacterial composition, reflecting the maturation of the gut microbiota in the children not exposed to neonatal antibiotics. The subjects exposed to neonatal antibiotics also demonstrated an expansion of bacterial establishment over time but in a distinct and less organized pattern. The infants exposed to neonatal antibiotics exhibited significantly lower gut microbiota richness as compared to the non-exposed infants at the age of 1 month. Interestingly, at the age of 6 months, the antibiotic-treated infants reached the bacterial richness level of the control infants, and at the ages of 12 and 24 months, the antibiotic-treated subjects gained significantly higher levels of bacterial richness as compared to the control subjects.

These results from a limited number of infants are consistent with previous reports (Arboleya et al., 2015; Fouhy et al., 2012; Tanaka et al., 2009), which have indicated a reduced abundance of bifidobacteria up to the age of 90 days in infants exposed to neonatal antibiotics. *Bifidobacterium* species are highly abundant throughout infancy, especially during lactation, and significantly decrease thereafter as infants are weaned and the diet expands to solid food (Arrieta et al., 2014). The importance of bifidobacteria is acknowledged, and lack of bifidobacteria has been shown to be associated with systemic inflammation and immune dysregulation early in life (Henrick et al., 2021).

As expected, taxonomic analysis revealed that bifidobacteria accounted for approximately 50% of total sequences at both 1 and 6 months of age, declining to around 25–30% at 12 and 24 months in infants not exposed to neonatal antibiotics. The relative abundance of bifidobacteria was consistently lower in the infants

exposed to neonatal antibiotics as compared to the control infants, except for the 6-month time point post-treatment. Feature-level analysis showed that the *Bifidobacterium* genus was composed of five unclassified features. The antibiotic-treated infants were shown to be less diverse in fecal *Bifidobacterium* features than the control at all four time points. For instance, the increase in *Bifidobacterium* in the antibiotic-treated group at 6 months was driven primarily by a single feature (unclassified1), whereas features termed unclassified2 and unclassified3 were predominantly found in the control infants. The differences between the other bacterial genera can be observed in Fig. 12c which summarizes the most significant shifts in the gut microbiota composition between the antibiotic-treated and control subjects at each timepoint.

The importance of bifidobacteria was also apparent in the study by Zhou et al., where 295 mother-child pairs were recruited to examine the association between intrauterine cumulative antibiotic exposure and infant growth, and also the potential role of neonatal gut microbiota in this association. The relative abundances of the phyla Actinobacteria and Firmicutes and order Bifidobacteriales were found to be significantly associated with weight and BMI growth speed in infants. In addition, in the same study, penicillin was positively associated with growth speed at the same age points. (Zhou et al., 2021) These findings suggest gut microbiota acting as mediator, although causation cannot be proved.

The present study extends these data with fecal sample follow-up until 24 months of age at which point a significant reduction in bifidobacteria was still evident. These data may be of clinical importance in light of recently published data suggesting that the specific individual gut microbiota and corresponding metabolomic profiles consolidate during the first two years of life (Vatanen et al., 2019). Our data, therefore, suggest that neonatal antibiotic exposure has a more profound and long-lasting impact on gut colonization than previously thought.

6.3 Gut Microbiota as a mediator

Perturbations of the gut microbiota resulting from antibiotic exposure provide a potential causal mechanism linking neonatal antibiotic exposure and reduced childhood growth. In **Study I** this was examined in an experimental model by using fecal microbiota transplantation (FMT) from antibiotic-exposed and non-exposed infants to germ-free (GF) mice.

During the 43-day follow-up period, male mice receiving FMT from infants one month after antibiotic treatment showed a notable decrease in relative weight gain compared to male mice receiving FMT from non-exposed infants. Conversely, the

growth of female mice remained unaffected by FMT using fecal samples obtained from antibiotic-exposed infants at one month of age. Notably, growth impairment in male mice persisted even when FMT was conducted with samples collected 24 months after antibiotic exposure. More importantly, the observed growth deviations resembled those found in humans.

These findings support the role of the gut microbiota as a biological mediator between early-life antibiotic exposure and altered growth trajectories. Indeed, the microbiota has been implicated as a mediator in various other disease processes, particularly in the context of antibiotics. The association between antibiotic exposure and gut microbiota alterations is being discussed earlier in this thesis, as well as the association between gut microbiota and various pathophysiological states. Epidemiological studies suggest modest associations between antibiotic exposure during early infancy and the risk of atopy, asthma, and eczema development (Ahmadizar et al., 2017; Kummeling et al., 2007; Penders et al., 2011; Räty et al., 2024), and perhaps stronger association with gut-associated diseases such as inflammatory bowel disease (IBD) (Örtqvist et al., 2019). In addition, changes in the specific taxa of fecal microbiota composition in the first weeks of life have been associated with the risk of atopic disease and asthma (Fujimura et al., 2016; Kalliomäki et al., 2001) as well as the development of overweight (Gao et al., 2015; M et al., 2008) later in childhood.

Can the gut microbiota in fact be considered a "mediator of obesity"? Zhao explored this topic in an interesting way already 10 years ago by using modified Koch's postulates (Zhao, 2013). These mechanisms can be studied in germ-free (GF) mice or after conventionalizing, where mice are made pseudo-GF by eliminating up to 90% of gut bacteria using antibiotic treatment. In a study by Cani et al, conventionalizing with ampicillin-neomycin combination prevented obesity despite a high-energy diet in mice (Cani et al., 2008). This reflects findings from GF studies, where a high-energy diet did not lead to weight gain in GF mice (Bäckhed et al., 2007). Zhao concluded that the available evidence, both from rodent and human studies, supports the hypothesis that gut microbiota with its compositional and specifically functional changes can initiate and aggravate metabolic diseases. The findings of this thesis support this opinion.

As randomized controlled trials are not ethically feasible in this context, much of the evidence supporting the hypothesis that early antibiotic exposure increases the risk of later-life obesity comes from experimental animal models. Human microbiota-associated rodent studies are considered the gold standard in assessing the possible causal role of microbiota alterations linked to human disease (Round & Palm, 2018; Staley et al., 2017). In order to establish causal relationships between altered microbiota and human diseases it has been used not only in obesity and malnutrition (Ridaura et al., 2013; Smith et al., 2013) , but also in various other

pathophysiological states, such as asthma, allergies, inflammatory bowel disease, Parkinson's disease, depression and autism spectrum disorder (Arrieta et al., 2015; Britton et al., 2019; Feehley et al., 2019; J. R. Kelly et al., 2016; Sampson et al., 2016; Sharon et al., 2019)

While evaluation of possible causation is mainly possible by rodent studies, it is notable, that the results must be interpreted with caution. In a systematic review by Walter et al., the researchers found that 95% of published studies on human microbiota-associated rodents reported significant results with pathological phenotype transfer to recipient animals. This high rate of transference was deemed implausible. (Walter et al., 2020)

Concerns have also been raised about the long-term effects of perinatal antibiotic exposure. Some studies have suggested that already fetal exposures to antibiotics can increase the risk for autism spectrum disorders. These effects could be explained by the actions of gut-brain-axis. In our cohort, this association could not be assessed due to sample sizing. However, Nitschke et al. reported that intrapartum antibiotic use was not associated with ASD risk, although prenatal exposure showed a slightly increased risk (RR 1.10), likely influenced by residual confounding. These findings should not alter clinical decision-making regarding necessary antibiotic use during pregnancy.(Nitschke et al., 2022, 2023)

Intrapartum antibiotic exposure has also been linked to increased risk of later autoimmune disease diagnosis. In a Finnish cohort of 45 575 vaginally born children, 21% were exposed to intrapartum antibiotics, and this was associated with a higher risk of autoimmune diagnosis (aHR 1.28, 95% CI 1.02-1.62). However, there was no detectable association with diagnosis of allergic or obstructive airway diseases. (Ainonen et al., 2024) In the same cohort population, intrapartum antibiotic exposure was associated with infectious diseases in early childhood, although the increased risk was statistically significant only at ages 7-28 days (adjusted incidence rate ratio [aIRR] 1.30, 95% CI 1.10-1.54) and 1-2 years (aIRR 1.10, 95% CI 1.02-1.18), indicating a relatively small effect size (Hakkola et al., 2024).

These findings support the idea that early-life programming – shaped by both intrauterine and extrauterine exposures – can predispose individuals to altered growth and disease risk. While modifying these early-life events is challenging, evidence suggests that postnatal intervention can still be effective. Encouragingly, a recent Swedish cohort study demonstrated that treatment of pediatric obesity significantly reduced the risk of morbidity and mortality in young adulthood (Putri et al., 2025), underscoring the potential for later-life interventions to mitigate early risks.

6.4 Sexual dimorphism

The effect of sex cannot be overlooked, although this study was not aimed to detect or evaluate sexual dimorphism. Interestingly, in **Study I** the neonatal antibiotic exposure was associated with reduced weight and height gain during the first six years of life in boys, but not in girls. Supporting this, the experimental study using GF mice demonstrated that FMT with fecal samples from antibiotic-exposed infants in the neonatal period results in growth failure in male, but not in female, mice. These data suggest a causal role for the antibiotic-induced gut microbiota perturbations in the pathogenesis of the growth impairment in antibiotic-exposed boys.

Previous epidemiological and experimental studies (Cox et al., 2014; S. A. Miller et al., 2018; Saari et al., 2015) have also indicated sexual dimorphism in the susceptibility to growth disturbances following early-life antibiotic exposure. This pattern was evident in our unselected study cohort, as boys were significantly more often exposed to neonatal antibiotics. Mode of delivery appears to interact with sex as well. For example, earlier studies have reported greater difference in growth rates from birth to 12 months of age between CS and VD in boys compared with girls (Mueller, Zhang, et al., 2019). Similarly, Bridgman et al found that male infants had a higher risk of overweight than girls, although uniquely only in those born by CS without the onset of labor (Bridgman et al., 2018). This in turn raises question of the antibiotic effect.

In a Danish study of 63 300 mother-child dyads examining the association of prenatal antibiotic exposure and birth weight, stratified analysis showed a significant association in preterm boys exposed to antibiotics, as their birth weight was higher than non-exposed. (Tomar et al., 2022) This association was not seen in term infants or in the overall cohort prior to stratification, further emphasizing the complexity of sex-related responses.

Biologically, boys are considered more vulnerable from early life onwards, which may contribute to the observation that more boys are born than girls (THL, 2024). Sex-based differences in disease prevalence, treatment outcomes, and responses to therapies are evident even in early childhood. Perinatal mortality is higher among boys compared to girls (Elsmén et al., 2004). Boys are more prone to prematurity and perinatal morbidity, such as respiratory distress syndrome (Aibar et al., 2012; Simchen et al., 2014). They also face a higher risk of intracranial hemorrhages and later neurological developmental disorders (L. A. Kelly et al., 2023). Girls, on the other hand, often exhibit a stronger immune response from an early age, which may help explain their lower risk of infections, whereas boys tend to experience more severe bacterial infections (Dias et al., 2022).

The underlying factors behind sexual dimorphism remain largely unclear, but emerging evidence suggests a link between hormonal and microbial activity. Early studies from the 1980s reported sex differences in neonatal progesterone. Among

term infants, boys had higher peripheral-blood concentrations than girls; among preterm infants, the pattern was reversed (Godo et al., 1981). More recently, Nuriel-Ohayon et al. describe in their study how progesterone increases the richness of several bacteria species, including *Bifidobacterium*. (Nuriel-Ohayon Cell 2019) Additionally, Abbas et al. (Abbas et al., 2022) have reported different growth patterns in male and female mice after obesogenic medication of olanzapine, adding up to hormonal effects.

Together, these findings underscore the importance of considering sex as a biological variable in studies on early-life exposures, particularly those involving the gut microbiota, and call for more mechanistic research to unravel the interplay between hormones, immunity, and microbiota in shaping long-term health outcomes.

6.5 Strengths and limitations

This study has several strengths which enhance the reliability of the results. The study population, SFBC, is an unselected, population-based cohort including all children born in the Southwest Finland between 2008 and 2010. The study design with serial standardized anthropometric measurements provides reliable growth data and enables an assessment of the age at which the BMI trajectories begin to diverge. However, these findings may not be generalizable across different settings and countries.

Another important strength is the uniform treatment protocol of suspected infection or sepsis. All children were evaluated and treated in a consistent manner, with uniform duration of neonatal antibiotic exposure across the empiric and infection groups, as presented in Table 2. The suspicion of neonatal infection or sepsis must initiate the use of antibiotics with a low threshold, and on the other hand, antibiotics must be stopped as early as possible, when bacterial infection is sufficiently ruled out. The impact of antibiotic exposure on neonates without symptoms, signs, or risk factors for infection remains unclear due to practical and ethical reasons. Differentiating between infection and antibiotic exposure is challenging, but this approach of empiric antibiotics without confirmed infection offers an optimal window to differentiate the effects.

Several limitations must be considered when interpreting these results. While the detailed data from the SFBC enabled differentiating between neonates briefly exposed to experimental antibiotic treatment and those who were diagnosed with infection, it is possible that our results have been confounding by the underlying causes leading to antibiotic exposure also in the subjects in whom infection was ruled out. It also has to be acknowledged that defining neonatal infection requiring full course of antibiotics is difficult, and despite of uniform treatment protocol, it is likely

that neonates with antibiotic exposure have had variable signs, symptoms and laboratory findings of infection.

In addition, the evaluation of gut microbiota was limited to stool samples, which primarily reflect the luminal microbiota of the distal colon. As a result, we were unable to characterize mucosa-associated communities or microbiota in other gut regions, where host–microbe interactions may differ or even be stronger, thereby limiting inference about site-specific effects.

Breast milk is a well-established determinant of infant gut microbiota composition and function, particularly affecting specific bifidobacteria. In the SFBC cohort, accurate breastfeeding data were unavailable, raising the possibility that variation in breastfeeding initiation, exclusivity, and duration among participants contributed to the observed alterations in the neonatal gut microbiota and the reduced bifidobacterial abundance among infants exposed to antibiotics.

However, the breastfeeding rates were available in the PEACHES cohort, which tracks 1707 children and their mothers in Bavaria (southern Germany), the University Hospital of Düsseldorf (western Germany), and parts of northern Germany. The study investigates the long-term effect of preconception maternal obesity on the development of overweight and associated metabolic diseases in mothers and their offspring. Unlike SFBC, PEACHES excluded women with pre-existing type 1 or type 2 diabetes and other chronic conditions before pregnancy. In addition, PEACHES is not a register-based study, and the recruitment process may have led to selection bias.

Nevertheless, no differences in breastfeeding rates during the first six months of life were observed between neonates with and without neonatal infection in the PEACHES cohort. Moreover, in this cohort, boys who experienced neonatal infection and antibiotic treatment exhibited significantly lower weight and height during the first five years of life, even after adjusting for breastfeeding. Additionally, all infants included in gut microbiota analyses and fecal microbiota transplantation (FMT) experiments in **Study I** were breastfed at one month, further reducing the likelihood of confounding at that time point.

Evaluation of neighborhood socioeconomic disadvantage in **Study II** is also a strength, as it was classified using objective measures such as household income, unemployment rate, and education level, with high geographical resolution. Finland's national population register ensures accurate residential mobility data, allowing for precise calculation of cumulative exposure to neighborhood disadvantage using regularly updated spatial information. In addition, the used statistical methods – marginal structural models with inverse probability weighting – correct for the differences in the baseline characteristics between included and

censored participants and minimize the potential of selection bias that could be introduced because of these differences. Sensitivity analyses using different neighborhood disadvantage cutoffs yielded consistent findings. In the main analysis, predefined cutoffs based on the total Finnish population were used to facilitate comparisons with other studies on the Finnish population.

Previous research on the associations of neighborhood socioeconomic status and childhood obesity has variably accounted for individual-level factors influencing obesity risk. In **Study II**, key confounders – single parenthood at the time of childbirth, smoking during pregnancy, prepregnancy obesity, and parental SES – were considered. Even after adjusting for these factors, the association between cumulative neighborhood disadvantage and childhood BMI z-score remained significant. However, paternal education and income data were unavailable, meaning some residual confounding is possible. Nevertheless, the strong correlation between parental SES and neighborhood disadvantage at birth suggests that major bias from unmeasured confounders is unlikely. Additionally, data on paternal BMI and smoking were not recorded.

Puberty onset is associated with both individual SES (Hiatt et al., 2017; James-Todd et al., 2010) and obesity risk (Busch et al., 2020; Deardorff et al., 2022), but unfortunately, this data was unavailable. However, given that follow-up ended at age seven – well before the typical onset of puberty – the observed associations are unlikely to be explained by differences in pubertal timing.

A notable limitation is the relatively high number of participants lost to follow-up. Only a small proportion of the missing data may be explained by the study subjects moving to geographical areas outside of Southwest Finland, and differences in data acquisition from electronic record systems in different municipalities are likely the reason for the missing anthropometric data.

Observational studies suggest that neighborhood socioeconomic disadvantage has broad health implications, including an increased risk of obesity in childhood, adolescence, and early adulthood, as well as diabetes in later life. These findings are often derived from direct comparisons of disease incidence between neighborhoods, raising concerns about health-related self-selection into residential areas. However, prior research indicates that selection bias is unlikely to fully explain the association between neighborhood disadvantage and health outcomes.

Experimental studies provide stronger evidence for a causal relationship between neighborhood characteristics and health. For example, the *Moving to Opportunity* experiment randomly assigned adults in disadvantaged US neighborhoods the opportunity to relocate to less disadvantaged areas. A follow-up 10–15 years later revealed lower obesity and diabetes rates among those who moved, compared to a control group that remained in disadvantaged areas. Similar findings

have been reported in natural experiments and self-controlled analyses examining changes in neighborhood conditions and health.

In **Study II**, several measures were taken to control for self-selection bias. First, comprehensive residential mobility data from birth to age seven allowed for precise control of relocation effects. Second, a broad range of confounding variables potentially affecting selection of place of residence, including single parenthood, immigrant background, smoking, obesity, maternal health conditions, and parental SES, were accounted for. While participants lost to follow-up were more likely to live in affluent areas and had lower BMI z-scores, these predictors of censoring were controlled for in the marginal structural model (MSM) analysis. Thus, selective retention is unlikely to be a major source of bias in this study.

6.6 Clinical implications and future perspectives

In this study, I demonstrate that antibiotic exposure – in a dose-response manner – and the neighborhood socioeconomic environment influence child growth, and that antibiotics induce alterations in the gut microbiota. However, the most important finding is the role of the gut microbiota as a potential mediator of these effects. While the associations observed are statistically robust, their clinical relevance in everyday life is likely to be modest. For instance, early antibiotic exposure was associated with a 0.15–0.2 z-score difference in growth, corresponding to approximately 250–300 grams at six years of age. At the population level, such a difference is unlikely to be noticeable. More importantly, the findings highlight a potential link between antibiotic exposure, microbiota alterations, and subsequent growth impairment.

These results align with growing evidence that the gut microbiota is closely linked to human health and that antibiotics can profoundly reshape its composition and function. Understanding these processes is relevant not only for child growth but also for public health, as microbiota disruption may increase infection risk and contribute to antimicrobial resistance. The microbiota's response to antibiotics is highly variable and influenced by multiple factors, including host characteristics, diet, infection burden, and environmental exposures.

An important dimension is the type and duration of antibiotic treatment. Broad-spectrum antibiotics typically cause more profound and long-lasting disruptions of the gut microbiota than narrow-spectrum agents, which target fewer bacterial groups. Repeated or prolonged treatments may further delay microbiota recovery and compound effects on nutrient absorption, immune maturation, and growth trajectories. The timing of exposure also matters. Early childhood represents a critical window when the gut microbiota is still developing and establishing stable ecological networks. Antibiotic perturbations during this sensitive period may therefore have more pronounced and long-lasting consequences than similar

treatments at older ages, when the microbiota is more resilient. Future studies should carefully evaluate age at exposure as a key modifier of antibiotic–microbiota–growth associations.

A further consideration is how to reduce unnecessary antibiotic exposure in the most vulnerable period of life. In neonates, clinical suspicion of infection often leads to empirical antibiotic treatment, even though only a minority of these infants ultimately have confirmed bacterial infection. Advances in early diagnostic methods – such as rapid molecular testing, host-response biomarkers, or integrated risk prediction models – may allow infections to be identified more accurately and earlier. By improving diagnostic precision, it may be possible to initiate treatment quickly in infants who truly need it while safely reducing antibiotic use in those who do not, thereby limiting disruption to the developing microbiota.

Social determinants also warrant greater attention. In this study, lower neighborhood socioeconomic status was associated with adverse growth outcomes, highlighting how disadvantage accumulates across early life. Current interventions are often focused on supporting pregnant women, for example through improved prenatal care and maternal nutrition. While these strategies are valuable, the present findings suggest that cumulative disadvantage continues to shape child health after birth. More precisely targeted interventions – not only at the individual level, but also regionally – may be needed to interrupt these trajectories and reduce inequalities in child growth and health.

From a translational perspective, strategies to support the microbiota during and after antibiotic treatment remain underdeveloped. Targeted interventions, such as engineered probiotics and fecal microbiota transplantation, are promising but experimental, and their safety and feasibility in children must be carefully evaluated. Identifying which interventions are most effective in restoring microbiota function after different antibiotic regimens, and at different developmental stages, will be an important step toward clinical translation.

Finally, individual susceptibility requires closer attention. Genetic background, nutritional status, and immune development likely shape how children respond to antibiotics and how well their microbiota recovers. Advances in precision medicine, where host, microbial, and environmental factors are jointly considered, may help identify vulnerable subgroups and guide tailored interventions.

In summary, while the direct impact of antibiotics on growth is modest, the broader implications for child development and long-term health should not be underestimated. Emphasis should be placed on clarifying how antibiotic type, duration, and age at exposure shape microbiota and growth trajectories, and on reducing unnecessary exposure through improved neonatal diagnostics. Equally important is to address the role of socioeconomic disadvantage not only during pregnancy but also throughout early childhood.

7 Summary/Conclusions

Based on the results presented in this thesis, the following conclusions can be deduced:

1. Early exposures, such as neonatal antibiotic exposure and early childhood socioeconomical status are associated with growth. It is possible, that these exposures act in a dose-response manner, and the effects may vary by age at the time of exposure.
2. Antibiotic exposure in perinatal period modifies gut microbiota. Although the quantifiable effects may be short-term, there are potentially long-lasting consequences.
3. The gut microbiota has a potential mediating role in the association between early antibiotic exposure and childhood growth.

There are also important clinical and socioeconomical issues beyond the primary aims of this thesis:

- The need for perinatal antibiotics should always be carefully evaluated, and better ways to rule out those who won't benefit has to be searched.
- Neighborhood and built-environment planning can be taken into consideration as part of efforts to mitigate socioeconomic disadvantage, in view of its association with health disparities.

Overall, in this thesis I propose that the gut microbiota acts as an intermediary factor between early environmental determinants and growth, and the effects of early-life exposures, no matter how short-term, have long-lasting effects.

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