Linear growth faltering in infants in low- and middle-income countries: the intestinal microbiota, the role of antibiotics, and the timing of linear growth failure.

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1. ABSTRACT

Stunting malnutrition in early life, defined as suboptimal linear growth, affects greater than one fourth of children under 5 years old in low- and middle- income countries (LMICs). Linear growth restriction largely accrues during the 1,000 day period from conception to 24 months of age and has long-term negative effects on child physical and mental development. However, variability in the timing of growth faltering during this period has not been investigated. In addition, nutritional strategies to improve linear growth and related outcomes in children have only had modest impacts, reflecting our limited understanding of what causes stunting. A largely unexplored determinant of infant growth is the ecosystem of microbes in the human gut, termed the microbiota. Animal models have shown that the gut microbiota can induce changes in weight. Growth gains have also been observed with antibiotic use that may result from antibiotic induced changes in gut microbiota composition and function. However, the gut microbiota has not been investigated as a determinant of linear growth. The objectives of this thesis were: (1) to identify linear growth trajectories into which HIV-unexposed infants, from LMICs, fall from birth to their second birthday and the socio-demographic and epidemiological factors that are associated with each growth trajectory; (2) to determine whether antibiotic treatment leads to improvements in growth in LMICs, determine the magnitude of growth improvements, and identify moderators of this treatment effect; and (3) to determine changes in the gut microbiota that are associated with linear growth.

To address Objective 1, I performed a secondary analysis of data from the Zimbabwe Vitamin A for Mothers and Babies trial. I applied *k*-means clustering for longitudinal data on a subset of 3,338 HIV-negative mothers and their infants followed-up at ten time points from birth until 24 months of infant age to identify linear growth trajectories, and multinomial regression to identify covariates associated with each trajectory group. Five distinct growth patterns were identified. These trajectories were all characterised by worsening linear growth restriction, but varied in the timing and steepness of growth declines. Maternal height and education, infant birthweight, and male infant sex were associated with different growth trajectories.

To address Objective 2, I performed a systematic literature review and meta-analysis of randomized controlled trials (RCT) of antibacterial use, conducted in an LMIC, in which growth was measured as an outcome. I pooled data from ten RCTs representing 4,316 children. In random effects models, antibiotic use increased both height and weight. In a sub-group analysis restricted to infants <24 months of age, similar treatment effects of antibiotics were observed. However, the effect on height was not statistically significant.

To address Objective 3, I performed secondary analyses of data from two twin cohorts of children 0-36 months old from Malawi and Bangladesh to identify gut bacteria associated with linear growth. The gut microbiota is a dynamic ecosystem of microorganisms that interact in a range of beneficial or antagonistic relationships. The structure of these relationships can be modelled as a network. Disruptions in these network relationships can point to taxa that are critical to growth faltering. I applied statistical learning and network analysis methods to identify and interpret changes in graphical models of microbiota covariance patterns to study stunted infants. I determined associations between microbiota members, implicated by network disruptions, and future linear growth by fitting longitudinal between-within twin regression models. Results suggested that overgrowth of *Acidaminococcus*, a genus of bacteria with high covariance network connectivity, may contribute to future growth deficits.

This thesis contributes to our understanding of linear growth faltering and points to areas for future research. The determinants of linear growth patterns suggest that infant growth may be predominantly determined by maternal characteristics and intrauterine growth. Antibiotics have a growth-promoting effect in human children that may be mediated by treatment of clinical or sub-clinical infections, or possibly by modulation of the intestinal microbiota. The infant gut microbiota may be a previously unrecognized determinant of linear growth deficits in resource-poor settings. Defining the mechanisms that underlie these findings will be important to inform optimal and safe approaches to achieving healthy growth in vulnerable populations of children worldwide.

RÉSUMÉ

La malnutrition qui retarde la croissance pendant l'enfance, dite croissance linéaire sous-optimale, affecte supérieure à un quart des enfants âgés de moins de 5 ans dans les pays à revenus faibles et intermédiaires (PFR-PRI). Le retard de croissance linéaire en grande partie attribué aux 1 000 premiers jours de vie, de la conception à l'âge de 24 mois, a des effets néfastes sur le développement physique et mental de l'enfant à long terme. Cependant, la chronologie de variabilité du retard de croissance sur cette période n'a pas encore été étudiée. De plus, les stratégies nutritionnelles pour améliorer la croissance linéaire et les finalités connexes chez les enfants n'ont eu que des effets modestes, reflétant notre compréhension limitée de ce qui provoque le ralentissement de la croissance.

Un facteur déterminant largement inexploré de la croissance infantile reste le microbiote intestinal humain. Des modèles animaux ont montré que le microbiote intestinal peut induire des variations de poids. Des gains de croissance ont aussi été observés avec l'utilisation d'antibiotiques pouvant résulter de changements induits par les antibiotiques sur la composition et la fonction du microbiote intestinal. Toutefois, le microbiote intestinal n'a jamais été étudié en tant que facteur déterminant la croissance linéaire.

Les objectifs de cette thèse ont été : (1) d'identifier les trajectoires de croissance linéaire d'enfants (non exposés au VIH et venant des PFR-PRI) qui s'effondraient entre l'âge de 0 et 24 mois, et les facteurs sociodémographiques et épidémiologiques qui sont associés à chaque trajectoire de croissance; (2) de déterminer si un traitement antibiotique pouvait conduire à l'amélioration de la croissance dans les PFR-PRI, de déterminer l'ampleur des améliorations de la croissance et d'identifier les modérateurs de cet effet de traitement; et (3) de déterminer les changements dans le microbiote intestinal qui étaient liés à une croissance linéaire.

Pour remplir le 1er objectif, j'ai effectué une analyse secondaire de données provenant du projet Zimbabwe Vitamin A for Mothers and Babies project (ZVITAMBO). J'ai appliqué le partitionnement de données longitudinales en k-moyennes sur un sous-ensemble de 3 338

mères séronégatives et leurs bébés à 10 moments t de l'âge des bébés entre 0 et 24 mois pour identifier les trajectoires de croissance linéaire et les régressions multinomiales pour découvrir les covariables liées à chaque groupe de trajectoires. Cinq formes de croissance distinctes ont été identifiées. Ces trajectoires étaient toutes caractérisées par une aggravation du retard de croissance linéaire, mais variaient en temps et en taux de retard de croissance. Les facteurs de taille et de l'éducation de la mère, de poids de naissance du bébé, et des bébés de sexe masculin entraînaient des trajectoires de croissance différentes.

Pour remplir le 2^{e} objectif, j'ai effectué une recherche bibliographique systématique et une métaanalyse d'essais cliniques comparatifs et randomisés (ECR) sur l'utilisation antibactérienne, menée dans un PFR-PRI, dans lesquelles la croissance a été mesurée en tant que résultat. J'ai réuni des données de dix ECR représentant 4 316 enfants. Dans les modèles à effets aléatoires, l'utilisation d'antibiotiques a permis d'augmenter la taille et le poids. Dans une analyse de sous-groupe restreinte aux enfants âgés de <24 mois, des effets similaires de traitement d'antibiotiques ont été observés. Cependant, l'effet sur la taille n'a pas été statistiquement significatif.

Pour remplir le 3e objectif, j'ai effectué des analyses secondaires de données provenant de deux cohortes doubles d'enfants âgés de 0 à 36 mois provenant du Malawi et du Bangladesh pour identifier les bactéries intestinales associées à la croissance linéaire. Le microbiote intestinal est un écosystème dynamique de micro-organismes qui interagissent dans une gamme de relations bénéfiques ou antagonistes. La structure de ces relations peut être modélisée comme un réseau. Les perturbations dans ces relations de réseau peuvent indiquer des taxons qui jouent un rôle essentiel dans le retard de croissance.

J'ai appliqué des méthodes statistiques d'apprentissage et des méthodes d'analyse de réseau afin d'identifier et d'interpréter les changements dans les modèles graphiques des schémas de covariance microbiotique pour étudier les enfants touchés par un retard de croissance. J'ai déterminé des liens entre des bactéries du microbiote, impliquées dans les perturbations du

réseau, et une croissance linéaire future en plaçant les modèles de régression longitudinale entre/dans les modèles de régression double. Les résultats suggèrent que la surcroissance d'Acidaminococcus, un genre de bactéries doté d'une haute connectivité de réseau de covariance, pourrait contribuer à un futur retard de croissance.

Cette thèse contribue à mieux comprendre le retard de croissance linéaire et ouvre de nouvelles pistes pour de futures recherches. Les facteurs déterminant les formes de croissance linéaire suggèrent que la croissance infantile peut être principalement déterminée par des caractéristiques maternelles et par la croissance intra-utérine. Les antibiotiques ont des effets favorisant la croissance chez l'enfant qui pourrait se faire par le traitement d'infections cliniques ou sous cliniques, ou éventuellement, par modulation du microbiote intestinal. Le microbiote intestinal des bébés peut être un facteur déterminant — qui n'a pas été pris en compte jusque-là — du retard de croissance linéaire dans les milieux pauvres en ressources. Il sera primordial de définir les mécanismes sous-tendant ces résultats pour communiquer les approches les meilleures et les plus sûrs pour donner une croissance saine aux enfants vulnérables du monde entier.

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Several years ago, while searching for potential PhD programs, I came across the website of Dr. Amee R. Manges, who at the time was at McGill University. I was looking for a training opportunity that would allow me to work at the interface of Basic Science and Epidemiology, on a project with relevance to Global Health. After several email correspondences and a few telephone conversations, Amee decided to take a chance on an unknown (me), and has since provided unrelenting support to me in my academic career. She has steered and guided me on this challenging, and often solitary, research path between the disciplines of Microbiology and Epidemiology. She has provided every resource available to her to allow me to become the investigator I aspire to be, working in the populations I am most passionate about. I also extend my heartfelt gratitude to Dr. Erica EM Moodie. Erica's dedication and commitment could not be made more obvious. Working between Montreal and Vancouver has, at times, been challenging, but I have never felt that her invaluable insights and suggestions were out of reach. I also thank Dr. David A. Stephens. He has been a great resource in the areas of my thesis that have perhaps posed the greatest methodological challenges to me, in that they are very far outside the typical Epidemiologists' "toolbox".

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3. CONTRIBUTION OF AUTHORS

1) Linear Growth Trajectories in Zimbabwean Infants.

E Gough, A Prendergast and A Manges developed the study concept and rationale, and formulated the research question. J Humphrey provided data. **E Gough**, E Moodie, A Prendergast, and A Manges contributed to study design and data analysis. **E Gough** performed the data analysis, data interpretation and drafted the manuscript. All authors read and revised the manuscript critically for important intellectual content and approved the final version to be published.

2) The Impact of Antibiotics on Growth in Children in Low and Middle-income Countries: A systematic review and meta-analysis of randomized controlled trials.

E Gough, J Humphrey and A Manges formulated the research question and rationale for the study. **E Gough**, E Moodie, R Stoltzfus, and A Manges provided input on study design and data analysis. **E Gough** and S Johnson conducted the literature search, study selection, and data collection. A Prendergast adjudicated study selection. A Prendergast, D Gibb, AS Walker, I Trehan, R Goto, S Tahan, and M Morais provided individual patient data, and provided insight into trial design, implementation, and database structure. **E Gough** performed the data analysis, data interpretation and drafted the manuscript. All authors read and revised the manuscript critically for important intellectual content and approved the final version to be published.

3) Linear growth faltering in infants is associated with *Acidaminococcus sp.* and community-level changes in the gut microbiota.

E Gough, J Humphrey and A Manges developed the rationale for the study. **E Gough** and A Manges formulated the research question and identified data sources. **E Gough**, D Stephens, E

Moodie, A Prendergast, R Stoltzfus, and A Manges provided input on study design and data analysis. **E Gough** performed the data analysis, data interpretation, and drafted the manuscript. All authors read and revised the manuscript critically for important intellectual content and approved the final version to be published.

4. STATEMENT OF ORIGINALITY

The research presented in this thesis represents original academic scholarship, primarily developed and executed by me.

In Manuscript One, I report the first study to classify individual linear growth profiles over time as a stand-alone outcome in an LMIC infant population. This is the first manuscript to describe variability in the timing of infant linear growth faltering in developing countries. It is also the first to provide evidence that faltering can be severe and dramatic even among infants who initially show acceptable, healthy growth. It also provides further evidence for the importance of maternal characteristics and intrauterine growth for infant growth.

In Manuscript Two, I report the first systematic literature review and meta-analysis of the impact of antibiotic use on growth in communities of predominantly malnourished children in low- and middle-income countries. This manuscript has contributed to the debate surrounding the role of antibiotics in community-based management of malnutrition, and the need for a better understanding of the mechanisms of action, and the costs and benefits of their use.

In Manuscript Three, I provide the first evidence of a relationship between the intestinal microbiota and linear infant growth restriction. This manuscript points to previously unrecognized mechanisms through which the community of microbes in the infant gut may impact linear growth, and provides direction for more focused investigations.

While I have received guidance and feedback from my co-supervisors, doctoral committee members, and co-authors on methodology, statistics and substantive knowledge, the studies presented in this thesis are my original work.

5. LIST OF ABBREVIATIONS

95%CI	95% Confidence Interval
AD	Aggregate Data
CH	Calinski-Harabatz Criterion
cm	Centimeters
DNA	Deoxyribonucleic Acid
EBF	Exclusive Breastfeeding
g	Grams
GDP	Gross Domestic Product
HAZ	Height-for-weight z-score
HR	Hazard Ratio
IPD	Individual Patient Data
IQR	Interquartile Range
kg	Kilograms
KML	*k*-means Clustering for Longitudinal Data
LAZ	Length-for-age z-score
LBW	Low Birthweight
L:M	Lactulose:Mannitol
LMIC	Low- and Middle-Income Country
MBF	Mixed Breastfeeding
MUAC	Mid-upper Arm Circumference
mm	Millimeter
OR	Odds Ratio
PBF	Partial Breastfeeding
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
RR	Risk Ratio
SAM	Severe Acute Malnutrition

SGA	Small-for-gestational-age
WASH	Water, Sanitation & Hygiene
WHO	World Health Organization
WHZ	Weight-for-height z-score
ZVITAMBO	Zimbabwe Vitamin A for Mothers and Babies

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8. INTRODUCTION

Undernutrition was responsible for 3.1 million deaths in children five years old or younger in low- and middle-income countries (LMICs) in 2011 (1). Linear growth faltering, in particular, is an indicator of chronic insults to childhood development and nutrition. Often measured in terms of deviations in attained growth from an age- and sex-matched reference population median (2), children with a length or height growth deficit greater than two standard deviations below the reference median are termed stunted. Stunting malnutrition affects greater than one fourth of children in the age group five years old or younger (1), and is associated with increased morbidity and mortality, and with poor cognitive, educational, and economic outcomes in later childhood and adulthood (3). Reducing the prevalence of stunting is a global health priority, and remains a target of global efforts set by the World Health Assembly (4). However, several countries are predicted to be unlikely to reach these targets if current trends in stunting prevalence and population growth persist (5). Estimates suggest that existing interventions, which include zinc supplementation and complementary feeding, would only reduce stunting by 20% if scaled up to reach 90% of children (6).

Deficits in linear child growth largely accrue from conception to 24 months of age (7), corresponding to a period in which factors such as maternal nutrition, feeding practices, dietary quality and quantity, and infections pose the greatest threat to child growth (8). However, how the timing and severity of linear growth restriction may vary between individuals during this period and the determinants of growth patterns have not been investigated. In addition, the modest impact of the most evidence-based interventions for stunting reflects our limited understanding of the underlying causes of linear growth failure. There is a clear need to better understand linear growth faltering in order to identify more effective and appropriately timed strategies for treatment and prevention of child stunting malnutrition.

A growing body of evidence suggests that the ecosystem of microbes in the human gut, termed the microbiota, is essential to regulating the inflammatory response and immune homeostasis

in the gut; performs important roles in nutrient harvesting and absorption from the diet; and protects the gut from invasion by pathogens (9,10). Colonization of the infant gut begins at birth, with the microbiota of the mother providing the earliest source of colonizing bacteria. Gut microbiota development continues through a process of microbial succession until the age of two or three years when an adult-like microbiota is established (11–13). Animal models have shown that the intestinal microbiota has a causal impact on weight gain (14,15). However, evidence for a relationship between the human gut microbiota and linear growth is lacking.

Growth gains have also been observed with antibiotic use in both humans and animals. Several studies have shown that antibiotics impact gut microbes (16,17). Microbiota recovery following antibiotic treatment is not always complete (18,19). A prevailing hypothesis for the growth gains associated with antibiotics is that antibiotic use alleviates infection or alters the composition and function of the intestinal microbiota. Systematic literature reviews and meta-analyses of other interventions that directly impact the gut microbiota, such as probiotics and prebiotics, have found significant growth gains in infants (20,21). Growth benefits have also been found in meta-analyses of water, sanitation, and hygiene (WASH) interventions in children, which indirectly impact the microbiota through prevention of exposure to pathogens and environmental microbes (22). However, antibiotics have the greatest documented impact on microbiota composition, and therefore provide the strongest evidence base to suggest a potential growth benefit of therapies that can modulate the gut microbiota. However, the effects of antibiotics on growth in humans have been inconsistent (23).

The objectives of this thesis were: (1) to identify linear growth trajectories into which HIV-unexposed infants fall from birth to their second birthday and the socio-demographic and epidemiological factors that are associated with each growth trajectory; (2) to determine whether antibiotic treatment leads to improvements in growth in LMICs, determine the magnitude of growth improvements, and identify moderators of this treatment effect; and (3) to determine changes in the gut microbiota that are associated with linear growth.

9. LITERATURE REVIEW

9.1. THE GLOBAL BURDEN OF CHILDHOOD MALNUTRITION

Undernutrition in early childhood consists of sub-optimal fetal, linear, and ponderal growth, as well as deficiencies in vitamin A and zinc, and suboptimal breastfeeding (6). These nutritional deficiencies underlie 45% of mortality in children under five years old worldwide, accounting for 3.1 million child deaths in 2011. Globally, an estimated 165 million children suffer from chronic undernutrition, measured in terms of attained height or length (linear growth), and 52 million suffer from acute undernutrition, measured in terms of attained weight (ponderal growth) (1). Z-scores are a common measure used to characterise nutritional status in infants and children. Children whose height-for-age z-score (HAZ) (i.e. deviations in attained height from an age- and sex-matched reference population median) lies more than two standard deviations below the median are termed *stunted*. Children whose weight-for-height z-score (WHZ) (i.e. deviations in attained weight from a height- and sex-matched reference population median) lies more than two standard deviations below the median are termed *wasted*.

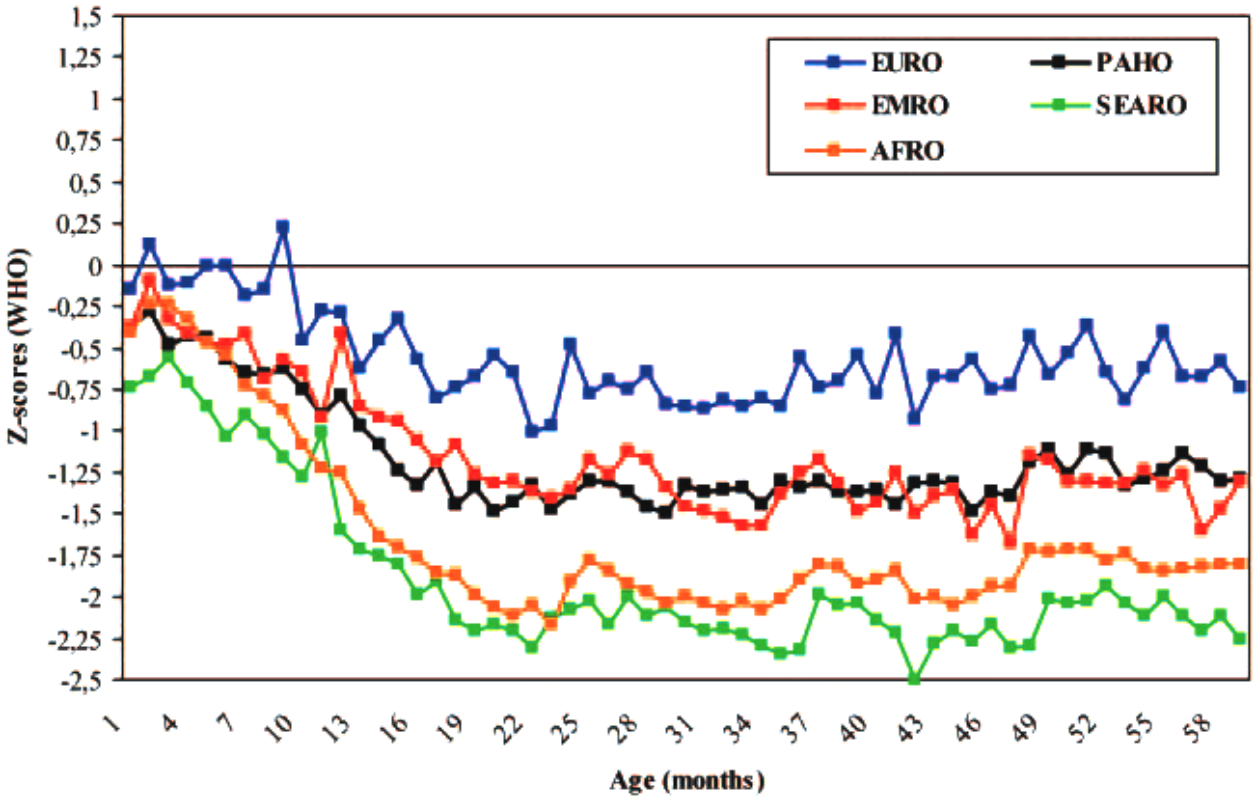
The World Health Organization (WHO) Child Growth Standard is a frequently used reference for growth in children younger than five years old. It characterises growth in infants across a number of countries, including LMICs, that were born to healthy, non-smoking mothers who lived in socio-economic conditions favourable to growth, and followed breastfeeding and complementary feeding recommendations (2). The WHO standard therefore represents expected child growth under optimal health conditions. Using this standard, more than a quarter of annual deaths in children five years old or younger are attributable to stunting and wasting combined. An estimated one million deaths are due to stunting and 875,000 are due to wasting (1).

The burden of stunting and wasting is greatest in LMICs, where 28% of children ≤5 years old are stunted and 8.8% are wasted. This is in stark contrast to 7.2% and 1.7% in high-income countries for stunting and wasting, respectively. In Africa and Asia in particular, the prevalence

of stunting (Africa: 35.6%, Asia: 26.8%) and wasting (Africa: 8.5%, Asia: 10.1%) in this age group are comparable to, or exceed, the global prevalence (25.7% stunted and 8.0% wasted) (1).

9-1. Figure 1. Mean Height-for-age Z-scores by Age, Relative to the WHO Standard, from Age 1-59 months.

Europe and Central Asia (EURO); Latin America and the Caribbean (PAHO); North Africa and the Middle East (EMRO); South Asia (SEARO); sub-Saharan Africa (AFRO).



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However, the global prevalence of childhood stunting (25.7%) is much greater than for wasting (8.0%) (1), and childhood linear growth shows larger deviations from the growth standard than ponderal growth (mean global HAZ and WHZ of approximately -1.75 vs -1.00 by age two years) (7). In sub-Saharan Africa and Asia, the regions most affected by stunting malnutrition, the average attained height decreases to almost two standard deviations below the reference

population median in the first two years of life (*Figure 1*). Although, there has been an estimated 2.1% annual decline in the global prevalence of child stunting malnutrition between 1990 and 2011 (1), these reductions have occurred predominantly in Asia. Stunting prevalence in Africa has shown more modest reductions, with increased population growth resulting in an increase in the absolute number of stunted children (1,5). In addition, while wasting appears to be reversible with adequate dietary intake and infectious disease prevention (24), the etiology of stunting is still poorly understood (8,25).

9.2. Consequences of Linear Growth Faltering for Adult Health and Human Capital

Stunting and wasting often occur together (26,27). However, while wasting tends to reflect short-term inadequacies in dietary intake; stunting is viewed as a consequence and marker of persistent insults to child health (8,24). Stunting increases morbidity and mortality rates in the short-term, but is also associated with poor motor and cognitive development, and reduced educational and economic attainment over the life-course (3,6,28). HAZ at two years of age is also inversely associated with adult systolic blood pressure (3), and shorter adult height is associated with cardiovascular disease, cardiorespiratory disease, and type 2 diabetes (12–17), suggesting additional consequences of stunting malnutrition for health during adulthood. Stunted mothers are at increased risk of having stunted children as well, creating an intergenerational cycle of restricted growth and development that hinders the developmental potential and human capital of entire societies (3,35,36).

9.3. The Socioeconomic Context of Linear Growth Faltering

The relationship between socioeconomic status and stunting prevalence in LMICs illustrates that stunting predominantly afflicts the poorest populations in the least developed countries (1). Analysis of household survey data on children 0-35 months old from 36 countries found that a 5% increase in per-capita gross domestic product (GDP), adjusted for purchasing power parity, was associated with a 0.4% decrease in the odds of being stunted (OR=0.996, 95%CI:0.993-1.000) (37). Ecological analyses also found evidence for an association between a

larger percent reduction in stunting prevalence and an increase in per-capita GDP ($r = -0.2$, $p=0.07$).

National improvements in child nutritional status are largely related to improvements in female education, nutrition, access to antenatal care, infectious disease risk, social-equity, hygiene and sanitation. LMICs that have undergone socioeconomic development have shown reductions in childhood stunting prevalence. In Brazil, the national prevalence of stunting decreased from 59.0% in 1974 to 7.1% in 2007. The steepest decrease in prevalence was observed from 1996 to 2007, when accompanying reductions in the gap between the richest and poorest income quintiles with respect to purchasing power; access to education, health care, water and sanitation services; and reproductive health indicators also occurred (38). In China, increasing per-capita GDP from 1985 to 2010 corresponded with a 70% reduction in stunting prevalence (39). However, for many countries that have shown a trend towards decreased stunting prevalence since 1990, a large gap in nutritional status between the wealthiest and poorest populations still exists; or only the wealthiest quintiles have shown significant improvements (39,40).

The disparity in linear child growth associated with socioeconomic position has also been observed in high-income countries. Using maternal education as a measure of socioeconomic status, a large prospective cohort study in the United Kingdom found that socioeconomic differences in linear growth from birth to age ten years were predominantly explained by differences in birth length, suggesting that factors during pregnancy which relate to social status may be responsible for restricted child growth and development (41). These maternal factors may include risky behaviors (e.g. smoking), access to proper nutrition during pregnancy (35,41), assortative mating by socioeconomic position, or possibly epigenetic influences driven by environmental factors (41). Conversely, when children are removed from the setting in which they became stunted, and are placed in better living conditions (e.g. through adoption), improvements in linear growth are often observed (42,43). These analyses also highlight the importance of access to a more nutritious diet, improved sanitation and hygiene, and

decreased risk of infections in influencing whether children achieve their full developmental potential after birth. The importance of socioeconomic factors is pervasive throughout high- and low-income countries alike, and further demonstrates the importance of contextual factors and environmental exposures in child growth.

9.4. THE DETERMINANTS OF LINEAR GROWTH FALTERING

9.4.1. Birth to 6 months

Linear growth faltering can begin *in utero* and continues for at least the first two years of life after birth. The average HAZ of infants at birth in LMICs is -0.5 (7) and future growth is closely associated with growth in previous months. The risk and severity of linear growth faltering in six to 24 month old children is strongly predicted by the severity of faltering prior to six months of age (44,45). Interventions to prevent stunting are therefore required early in the life-course during this critical window of opportunity from conception to a child's second birthday (the first thousand days) (6).

Besides birth length, other common measures of birth size are birthweight, and weight for gestational age. These are often used as measures of fetal growth restriction (1). Infants born with a low birthweight (<2,500g), or who are below the 10^{th} percentile of weight for their sex and gestational age, are classified as low birthweight (LBW) or small-for-gestational-age (SGA), respectively (46,47). An estimated 11%-16% of infants in developing countries are born LBW (48,49), and 27% are born SGA (1). Infants with fetal growth resitrction show an increased risk of stunting during infancy (50–59). In an analysis of longitudinal data from 19 LMIC cohorts, the odds of stunting between 12 and 60 months of age was 2.9 times greater (95%CI: 2.56, 3.33) in infants born LBW and 2.3 times greater (95%CI: 2.12, 2.54) in infants born SGA after adjusting for age (50).

Preterm birth (≤37 weeks gestational age) is a contributing factor in small birth size, but is also independently associated with poor infant growth (50). Fetal growth restriction and preterm

birth often co-occur in LMICs, where an estimated 20% of preterm infants are also born SGA (1). Infants born SGA and preterm had a 4.5 times greater odds (95%CI: 3.42, 5.93) of stunting between ages 12 and 60 months, compared to full-term infants born at an appropriate size for gestational age (50). This effect was twice as large as the association between stunting and SGA, or preterm birth, individually.

During the period from birth to six months of age, linear growth rates peak in healthy infants (8). The WHO recommends exclusive breast feeding (EBF) during this period, defined as only breast milk and no other foods or liquids (60), because the benefits to morbidity, mortality and cognitive development are well established (48,60–62). However, while breast feeding promotion interventions have shown significant effects on improving EBF rates, evidence for benefits on nutritional outcomes is limited (6). Kangaroo maternal care improves breast feeding rates, but improvements in growth have only been observed in RCTs that targeted LBW infants (63). Evidence to support a growth benefit in healthy full term infants is scarce (64).

The nutritional status of the mother during pregnancy may be critical. Maternal nutritional indices, including short stature, low body mass index (BMI) and low weight gain during pregnancy, are associated with reduced birth size in infants (65–67). Measures of maternal weight during pregnancy reflect the overall adequacy of nutrient intake to meet maternal and fetal needs, as well as requirements for placental growth, development and function (65). Dietary supplementation to pregnant mothers has produced improvements in birthweight and SGA in newborns in a number of RCTs (68–72), and has also been shown to improve infant growth (73,74). Micronutrient supplementation with vitamin D or iron/folate during pregnancy reduced the risk of giving birth to a LBW infant by 52% (RR=0.48; 95%CI: 0.23 to 1.01) and 20% (RR=0.80; 95%CI: 0.71, 0.90), respectively (70,72); while calcium supplementation produced an 85 g gain (95%CI: 37g, 133g) in birthweight (71). Macronutrient supplementation with balanced protein energy intake during pregnancy reduced the risk of giving birth to an SGA infant by 31% (RR=0.69; 95%CI: 0.56, 0.85) (69). In terms of infant growth, multiple micronutrient supplementation during pregnancy reduced the rate of stunting by 27% (HR=0.73; 95%CI: 0.60,

0.87) at age 12 months, and increased height by 0.64cm (95%CI: 0.04, 1.25) at 6 to 8 years of age (73,74).

Maternal height is also strongly associated with size at birth and with stunting in children younger than five years old (36,58,59,75,76). An analysis of household survey data from 54 countries found a 1 cm increase in maternal height to be associated with a 3.2% reduction (RR=0.968; 95%CI: 0.967-0.968) in the risk of stunting in children younger than five years (75); and analyses of data from five LMIC birth cohorts indicate a 0.037 (95%CI: 0.033,0.040) increase in HAZ at age two years for every 1 cm increase in maternal height, after conditioning on birthweight and all previous height measurements (36). Short maternal stature reflects the stressful nutritional environment of the mother from conception and throughout life. Disturbances to fetal development may impair maturation of organ systems, and subsequently restrict a mother's capacity to deliver nutrients to her fetus when she reaches reproductive age (75). Short stature may also place physical constraints on fetal growth. Maternal height may also be an indicator of socioeconomic status, with shorter mothers being more likely to live in poorer conditions with access to less nutritious foods (36).

9.4.2. 6 months to 24 months

On average, linear growth deficits are largest from six to 24 months of age across countries (7). During this period, infants are introduced to solid foods and begin to explore their environments. Dietary deficiencies, infections and diarrhea are the risk factors most strongly and consistently associated with linear growth faltering during this period (1,77–80).

With respect to diet, currently available interventions focus on dietary supplementation from 6-24 months of age, but have only a modest benefit on linear growth. The Lancet Series on Maternal and Child Nutrition estimated that nearly one million deaths in children younger than five years old could be prevented if the coverage of evidence-based zinc supplemantation and complementary feeding interventions could be increased to reach 90% of children in 6-24

months old. However, the prevalence of child stunting would only be reduced by 20.3% (range 10.2% to 28.9%) (6).

Acute diarrhea and respiratory infections are commonly occurring illnesses in infants and children (80). Malabsorption of nutrients, increased nutrient loss during episodes of diarrhoea, gut inflammation, impaired intestinal barrier function, impaired nutrient metabolism and utilization, diversion of nutrients away from growth to support immune activation, and loss of appetite are possible reasons for impaired growth during infection (78–80). An analysis of longitudinal data from five LMICs found that the odds of stunting by age two years were 13% greater (95%CI: 1.07,1.19) for every five episodes of diarrhea experienced during that time (77). Another multi-country analysis found a 0.10 reduction in HAZ (95%CI: −0.10,−0.00) at age two years for every ten additional days of diarrhea per child-year of follow-up, and an average deficit of 0.38 cm (95%CI: −0.59 cm, −0.17 cm) at age two years associated with the typical diarrhea burden of 23 days of diarrhea per year (81). Diarrhea prevention has therefore been another target of interventions to restore linear growth deficits. However, analysis of data from seven LMIC cohorts found that linear growth from age six to 24 months was faster during periods without diarrhea, if preceded by a period in which diarrhea occurred. The investigators observed that children exhibited approximately 0.03 mm greater height growth per month (95%CI: 0.01 mm, 0.06 mm) in the diarrhea-free period for every 1% increase in diarrhea prevalence in the *previous* time period. This was in contrast to a reduction in the rate of height growth per month for every 1% increase in diarrhea prevalence in the *concurrent* time period (82). Because of the subsequent recovery from growth deficits incurred during episodes of diarrhea (82–85), the relative contribution of diarrhea to child stunting, and consequently, the potential impact of diarrhea prevention programs on child growth remains unresolved.

Furthermore, a number of studies have shown that inflammation and permeability in the small intestine are associated with poor linear growth (86–89). This sub-clinical gut pathology, which has been termed environmental enteric dysfunction (EED), is characterized by histological changes to the gut wall that result in reduced surface area and a ‘leaky’ gut. EED is

asymptomatic, and is acquired early in life among children living in unsanitary conditions (78,90–93). A common biological measure of intestinal permeability and gut absorptive capacity is the lactulose:mannitol (L:M) ratio, which assesses permeability to lactulose and absorption of mannitol (94). A larger L:M ratio indicates increased gut permeability and impaired nutrient absorption. An increase in L:M ratio is associated with a 0.10 decrease in HAZ in children less than five years old (89,95). An increase in fecal markers of intestinal permeability has also been associated with a reduced gain in HAZ (-0.05 standard deviations) over a six month period in infants from eight countries (87). Increased intestinal permeability enables translocation of bacteria or bacterial by-products across the gut wall to occur, leading to chronic systemic inflammation, reduced levels of insulin-like growth factor 1, and larger linear growth restrictions (96). This evidence points to the overall health of the infant gut as a potential determinant of chronic infant growth failure and poor development.

Other infections associated with stunting malnutrition include intestinal helminth infections and malaria. While intestinal helminth infections have been associated with child stunting in cross-sectional studies (97–99), the intensity and prevalence of intestinal helminth infections have been estimated to peak at 10-14 years and 14-21 years of age, respectively (100). The evidence for improved linear growth resulting from deworming in children aged 16 years or less is limited. Two systematic reviews and meta-analyses of RCTs found no significant improvement in height following deworming treatment in this age group (101,102). On the other hand, a recent RCT of deworming targeting infants younger than 24 months of age found a significant improvement in HAZ (103). With respect to malaria, a number of studies have identified an association with stunting in children (104–106), while others have not (107). A recent analysis of 1,070 infants, recruited at three months and follow-up until age two years, used Mendelian randomization and matching to control for unmeasured confounders and reported a 0.32 (95%CI: 0.09, 1.00) absolute increase in the risk of stunting for each additional episode of malaria (108). Another prospective cohort study of children less than 72 months old found reduced gains in height of 0.08 fewer cm (95%CI: -0.15 cm,-0.01 cm) over six months for each additional episode of malaria (109).

Finally, recent evidence suggests that changes in linear growth may be preceded by changes in weight (24,110,111). An analysis of data from eight longitudinal cohorts reported that a one standard deviation increase in WHZ in the previous six month period was associated with a 0.33 cm (95%CI: 0.11 cm, 0.54 cm) and 0.72 cm (95%CI: 0.52 cm, 0.92 cm) greater attained length at 18 and 24 months of age, respectively. Another analysis of data from four longitudinal studies of infants from birth to one year, found that WHZ in the previous 3-month interval was positively correlated with HAZ in the current interval, for all 3-monthly intervals investigated (r = 0.15 to 0.36) (111). Using a saltatory model of growth, in which brief spurts in growth punctuate longer periods of no growth, Lampl *et al.* showed that in infants followed-up from birth to one year of age, linear growth spurts were concurrent with (in both sexes), or preceded by (in male infants), growth spurts in weight (112). The mechanisms through which ponderal growth may facilitate later linear growth are unknown.

9.5. PATTERNS OF LINEAR GROWTH

Current understanding of the evolution of linear growth failure and recovery over time is largely based on analyses of cross-sectional data on attained growth, at each month of age, averaged across 54 predominantly LMICs (7). These analyses show that the average HAZ in LMICs is below the standard population median at birth, then rapidly declines to approximately -2 standard deviations by age 2 years, with little to no recovery by age five. However, aggregating cross-sectional data across countries in this way may obscure important differences in growth patterns between individuals within a population.

For example, the pattern and severity of linear growth faltering in the first 24 months of life varies by infant HIV-infection status and with the timing of HIV-infection. Among HIV-negative Zimbabwean infants, most growth faltering occurred from six to 21 months, with in-between periods of either stable growth (from six weeks to six months) or linear growth improvement (from 21 to 24 months). HIV-infected infants showed different linear growth patterns (113). In a study of institutionalized children less than two years old at study entry in Portugal, four

patterns of linear growth during nine months of follow-up were identified: a persistently small group (below the fifth percentile for height at each visit), a persistently high group (above the 75th percentile at each visit), a declining group, and an improving group. Factors associated with growth trajectory membership were birthweight, birth length, and age at institutional admission. Children in the persistently low group with no improvements over time were younger when they entered the institution (4.44 months ± 5.22) than the remaining three groups of children (114), suggesting that the age at which sub-optimal environmental conditions for growth begin, may influence the subsequent growth pattern.

Other studies show that children who are stunted early in life may later experience higher growth rates than their healthy, non-stunted peers, and "catch-up" to their original growth curves, even in the absence of nutritional intervention. Catch-up growth is often defined as greater HAZ, or a greater rate of growth than is expected for a child's age, occurring after a period of growth restriction (115), or as recovery from stunting (116). However definitions of catch-up growth and methods to determine the extent of catch-up vary (115–120). With this caveat, most studies have found evidence for catch-up growth during adolescence and adulthood, among persons who were stunted before the age of five (115,116,119–122). In an analysis of 1,252 Filipino children stunted at age two years, Adair *et al.* reported that 30.3% were no longer stunted by age 8.5 years, with an average gain in HAZ of 1.1 standard deviations. Of these recovered children, 11% could be considered fully recovered with a HAZ > -1.0 (116). However, these studies also suggest that the extent of catch-up growth may vary by socioeconomic status, sex, maternal stature, birth size, parity, and the severity of childhood stunting (115,116,119,120).

In LMICs, catch-up growth during infancy for newborns with restricted fetal growth may be limited. An analysis of 470 Malawian infants followed-up for a median 11 months after birth, found that LBW infants showed no evidence of catch-up in linear growth by 12 months of age (125). The lack of catch-up growth was attributed to an increased risk of further linear growth

faltering after birth, which was associated with birthweight, preterm birth, number of illness episodes during follow-up, and male sex.

The largest deficits in linear growth occur during the first one thousand days of life. However, the literature suggests that individual growth patterns with respect to growth restriction and recovery vary between individuals. During the period from conception through age two years, infants experience a number of maternal, dietary, and environmental exposures, including infections, that may also vary with infant age. However, the patterns of linear child growth, the timing of growth faltering, the possibility of recovery, and the determinants of these growth patterns are not well understood. Understanding the temporal patterns of linear growth in infants and the factors that determine these patterns is critical to the design of appropriate age-targeted interventions to promote healthy infant growth, and will inform strategies to prevent and manage child stunting.

9.6. THE INTESTINAL MICROBIOTA AND GROWTH

A number of studies have identified important correlates of child stunting malnutrition. However, the limited success of existing interventions reflects our poor understanding of the pathophysiology and etiology of stunting. This has important implications for countries' ability to achieve the World Health Assembly global target to reduce stunting by 40% by the year 2025 (4). There is a need to better define modifiable mechanisms that underlie stunting so that tractable pathways for intervention can be identified.

Four decades ago, Rosenberg *et al*. suggested the potential role of recurrent intestinal infections and altered or abnormal gut microbial ecology in child malnutrition (23). Within the human intestinal tract, trillions of microbes form an ecosystem, termed the microbiota, that contributes to health (126). The human gut is estimated to harbour approximately 10^{11}–10^{12} microbes per milliliter of luminal content (126), and more than 100 times the number of genes of the human genome (127). The intestinal microbiota functions in regulating immune system

activation and inflammation in the gut, in maintaining gut homeostasis (128,129), and in protecting the gut from invasion by pathogens (126). The intestinal microbiota also performs critical nutrient harvesting and absorption functions for the human host (9,10).

Advances in DNA and RNA sequencing technology have revolutionized our ability to quantify the composition of microbial communities that inhabit the body, and in the past few years, evidence for a role of the intestinal microbiota in child growth has emerged. Observational studies in humans (130–133) have demonstrated a relationship between the composition of the intestinal microbiotas of children and severe acute malnutrition (SAM), a form of severe wasting characterized by pitting oedema or WHZ ≤ -3 (134). A causal effect of the intestinal microbiota on weight has also been shown using experimental animal models. In one experiment, weight loss in mice resulted from transplantation of donor feces from children with SAM, but not their healthy twins (14); while increases in total body mass and fat mass were induced in mice transplanted with donor feces from obese adults, but not their lean twins (15). However, the specific changes in the microbiota that contribute to growth in humans remain unclear, and no studies to date have investigated the intestinal microbiota as a determinant of linear growth.

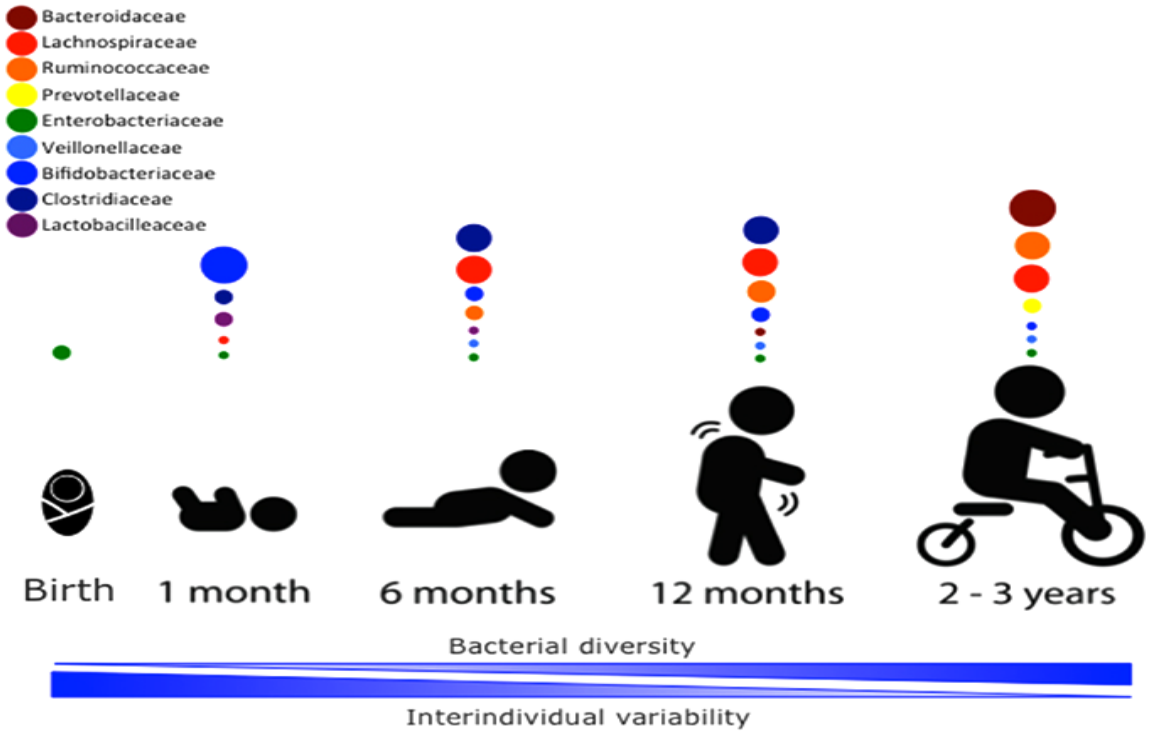
9.7. Infant Intestinal Colonization and Microbial Succession

The traditional view holds that the infant intestine is sterile at birth. It then undergoes an evolution in composition, including successional waves of colonization with developmentally appropriate bacterial species up to the age of 2-3 years. Immediately after birth, the infant gut is predominantly colonized by facultative anaerobic *Enterobacteriaceae* species of bacteria, which are able to grow in a low oxygen environment. In the first few days after birth, as the oxygen levels in the infant gut are depleted, the microbiota undergoes a shift to predominantly anaerobic microbes, which are able to thrive without oxygen.

The major initial sources of microbes for colonization of the infant gut at birth are the mother and the immediate environment. The infant gut microbiota is most similar to the mother's vaginal or skin microbiota in the few days after birth, depending on the mode of delivery (11–13). The gut microbiotas of vaginally delivered newborns appear to be most similar to the mothers' vaginal microbiotas, while the gut microbiotas of newborns delivered by ceasarian section more closely resemble the mothers' skin microbiotas and the surrounding environment (135). In addition to vaginal microbes, the mother's intestinal microbiota may also be transferred to the infant via the gastrointestinal tract during vaginal delivery (136,137). By age two years, infants delivered by ceasarian section show lower gut bacterial diversity, potentially due to late colonization with bacterial species they would have obtained from the mother during vaginal delivery (13).

After birth, infant diet largely determines the composition of the gut microbiota. In breastfed infants, *Bifidobacteria* species are the most abundant gut bacteria (138). Inoculation of the newborn with *Bifidobacteria* occurs via human breast milk. Human breast milk is rich in oligosaccharides, which *Bifidobacteria* species preferentially digest (139), demonstrating an evolutionary balance between infant nourishment and microbes designed to digest these nutrients (140,141). Formula fed infants on the other hand, show larger proportions of *Staphylococcus*, *Streptococcus*, *Enterobacteria*, and *Clostridium* species of bacteria (12,142–144).

9-2. Figure 2. Stages of Microbial Colonization of the Intestine.



Source: Arrieta et al., 2014 (13) Figure as originally published in Arrieta MC, Stiemsma LT, Amenyogbe N, Brown EM and Finlay B. The intestinal microbiome in early life: health and disease. Front Immunol 2014; 5:427. doi: 10.3389/fimmu.2014.00427 Copyright: © 2014 Arrieta, Stiemsma, Amenyogbe, Brown and Finlay. Reproduced under the terms of the Creative Commons Attribution License (CC BY) http://creativecommons.org/licenses/by/3.0/
No modifications to the Figure have been made.

The introduction of solid foods into the infant diet correlates with another major shift in gut microbiota composition. A wider variety of nutrients available in solid foods, many of which cannot be digested by the infant, corresponds with an increase in the abundance of *Bacteroides*, *Clostridium*, and *Ruminococcus* species, and a decrease in the *Bifidobacteria* and *Enterobacteriaceae* species that are predominant in the first six months. In the subsequent period from six months to 2-3 years of age, the infant microbiota develops a more adult-like composition, with increased bacterial diversity both in terms of the number of different types of bacteria present in the gut, and the evenness of bacterial abundance (11–13).

Microbiota development is very individual specific. Microbiota compositions within individual infants between adjacent time points correlate strongly. The initial colonizing bacteria and the subsequent evolving community structure play a pivotal role in how the composition of this microbial community in the gut develops during infancy (126,145). One recent study proposed a link between healthy gut microbiota development and SAM in infants (133). This study of infants in Bangladesh found that the abundances of 24 specific bacterial taxa in the infant gut were highly predictive of age in healthy, well-nourished infants. Using the same 24 age-discriminatory bacterial taxa, the investigators found that the predicted age of infants who suffered from SAM was lower on average than the predicted age of age-matched healthy infants (133). Based on these results, the investigators proposed that developmental delays in gut microbiota compostion may be associated with undernutrition.

There is growing interest in the role of the intestinal microbiota in infant growth and child nutritional outcomes due to its role in intestinal inflammation, protection from pathogens, nutrient harvesting and absorption in the gut. The development of the infant gut microbiota also correlates temporally with the period of greatest infant linear growth faltering. Understanding the role of the gut microbiota in infant growth may point to new strategies for the treatment and management of child stunting. Microbiota modulation has already been shown to be an effective treatment in some situations (e.g. intestinal infusion of whole stool from a healthy donor into patients with recurrent *Clostridum difficile* infection (146)).

9.8. Microbiota Modulating Interventions and Growth

A number of studies have found that antibiotics induce changes in gut microbiota composition (16,17). These changes are not always completely reversible (18,19). Recent work has shown that intestinal microbes may not return to their pre-treatment abundance levels, even after a single exposure to antibiotics (18,147,148). Antibiotics have also been used for their growth promoting effects in food animals since the 1940s. Small daily doses of broad spectrum

antibiotics have been found to improve average daily weight gain in farm animals (149–157). Animal trials have found as much as 73% greater average daily weight gain in treated compared to untreated livestock (150). In humans, antibiotics have also shown potential benefits in growth (23). This growth promoting effect has been observed in hospitalized infants (158), acutely malnourished infants (159), HIV infected children (160,161), HIV infected adults (162), and obese adults in developed countries (163). The potential beneficial effect of antibiotic use on human growth may result from the alleviation of clinical or sub-clinical infections. It has also been proposed that, given its direct effect on microbiota composition, antibiotic use may impact growth through alteration of the intestinal microbiota (164).

However, the effects of antibiotic use on growth in human trials are inconsistent. Other than antibiotic studies, few studies of microbiota altering therapies exist with which to investigate their potential growth promoting effects. Probiotics refer to microorganisms that are believed to provide health benefits when consumed, and prebiotics refer to carbohydrates that promote the growth of normally abundant, beneficial microbes in the human intestine. The impact of probiotics and prebiotics on intestinal microbiota composition have not been as clearly documented as for antibiotics (19); however, these studies provide another source of evidence for the possible impact of microbiota altering interventions on child growth. A recent individual patient data (IPD) meta-analysis of five RCTs found a significant gain in weight of 1.5g/day (95%CI: 0.09g, 2.93g) in infants fed formula milk containing the probiotic *Bifidobacterium lactis* compared to infants given control formula (20). This meta-analysis found no significant effect for height (0.02cm/month; 95%CI: -0.08cm, 0.11cm). Another meta-analysis of four RCTs comparing formula supplemented with fructo- or galacto-oligosaccharide prebiotics versus control formula found an average weight gain of 1.07g/day (95%CI: 0.14g, 1.99g) in full term neonates (21). This study did not report an effect for height. Finally, chronic exposure to environmental or pathogenic microbes resulting from conditions of poor sanitation and hygiene may result in altered gut microbiota composition and function, with subsequent negative effects on intestinal inflammation, permeability and child growth (78,90). A meta-analysis of RCTs of children younger than five years old who received a WASH intervention to reduce

pathogen exposure found a 0.08 standard deviation increase in HAZ (95%CI: 0.00 to 0.16) compared to control children (22).

Of the existing interventions that are believed to involve microbiota modulation as a possible mechanism for their impact on growth, antibiotics have the greatest documented impact on microbiota composition. If the microbiota does modify child growth potential, then it is reasonable to hypothesize that studies of antibiotic use and growth would provide the most compelling evidence for the growth benefit of therapies that can modulate the gut microbiota. Considering that existing interventions only modestly restore the growth deficit in children, it is important to investigate the antibiotic impact on human growth, and to determine possible explanations for the inconsistent growth benefits of antibiotics in human studies.

9.9. CONCLUSION

Overall, there is a clear need for a better understanding of linear growth faltering to inform interventions to prevent and treat child stunting malnutrition. On average, linear growth restriction primarily occurs in the period from birth to the infant's second birthday, with the largest deficits accumulating after age six months. However, the predictors of stunting evolve from the prenatal to toddler period, and evidence suggests that the timing and patterns of individual growth may vary as well. Understanding linear growth patterns is important for planning appropriately timed interventions to maximize growth benefits during this critical period. A potential unrecognized contributor to linear growth faltering is the intestinal microbiota. The timing of infant microbiota development coincides temporally with the critical window of growth failure. The gut microbiota is increasingly recognized as an important determinant of overall gut health and weight gain, both of which are important correlates of linear growth. Novel interventions to improve infant growth are also needed. Assessing interventions that involve microbiota alteration can provide an important proof-of-concept that microbiota-targeted therapies can improve child growth and nutrition.

10. Linear Growth Trajectories in Zimbabwean Infants.

10.1. Preface to "Linear Growth Trajectories in Zimbabwean Infants."

It is widely agreed that linear growth failure predominantly occurs before the age of two years. Very limited evidence exists, however, that provides insight into the temporal dynamics of linear faltering, or how patterns of growth vary across sub-populations of infants. A better understanding of the trajectories of growth experienced by infants and of the timing of growth failure and recovery will provide an important evidence base to design and implement interventions appropriate for specific target ages. In this manuscript, I investigate the timing of linear growth faltering and patterns of linear growth, in a resource-poor population, using a representative cohort of mother-infant pairs from Harare, Zimbabwe. Regression methods treat each infant's follow-up measurements as repeated observations of growth, and aim to estimate the average change in growth per unit of follow-up time, where change in growth over time is assumed to be linear. I apply *k*-means clustering for longitudinal data to identify groups of infants with similar non-linear growth patterns. Infants are then classified according to their membership in a group, characterized by a common growth trajectory, to determine predictors of infant growth. No other studies to date have applied longitudinal data clustering methods to investigate linear growth patterns in an LMIC setting. This manuscript provides important insights into the determinants of longitudinal growth in infants that can inform more age-targeted interventions to prevent childhood stunting.

10.2. MANUSCRIPT: "LINEAR GROWTH TRAJECTORIES IN ZIMBABWEAN INFANTS."

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Abstract

Background: Undernutrition in early life, measured in terms of poor growth, underlies almost half of all child deaths globally. Stunting malnutrition in particular, defined as suboptimal linear growth, has long-term negative effects on childhood development. Linear deficits largely accrue by 24 months of age (the first 1,000 days). Understanding the patterns of linear growth and the factors that determine growth trajectories during this period is critical to the timing of interventions to improve infant nutritional status. **Methods:** We performed a secondary analysis of data from the Zimbabwe Vitamin A for Mothers and Babies trial. We used longitudinal data collected at ten time points on a subset of 3,338 HIV-unexposed infants to investigate linear growth from birth through the infant's second birthday. We used *k*-means clustering for longitudinal data to identify linear growth trajectories, and multinomial regression to identify covariates associated with each trajectory group. **Results:** Five distinct

growth patterns were identified. These trajectories were all characterised by worsening linear growth restriction, but varied in the timing and steepness of growth declines. In our multivariable model, maternal height (OR: 1.06, 95%CI: 1.03, 1.10), education (OR: 1.10 ,95%CI: 1.01,1.21), infant birthweight (OR: 1.17, 95%CI: 1.09, 1.27) and male sex (OR:1.18, 95%CI:1.09,1.27) were associated with the least growth restricted infants (Group A). Infant birthweight (OR**:** 0.87, 95%CI: 0.79, 0.94) and male sex (OR: 1.97, 95%CI: 1.32, 2.94) were associated with the most severely growth restricted infants (Group E). **Conclusions:** Longitudinal infant growth trajectories may be predominantly determined by maternal characteristics and intrauterine growth.

Introduction

Undernutrition in early childhood underlies 45% of all mortality in children aged under five years worldwide, resulting in 3.1 million deaths annually (1). Linear growth faltering in children is viewed as an indicator of long-term nutritional status and is often measured in terms of z-scores (2). Children whose linear growth, measured as length- or height-for-age z-scores (LAZ or HAZ), is more than two standard deviations below a reference population median are termed stunted. In addition to its short-term effects on morbidity and mortality, stunting also contributes to poor motor development, cognition, educational achievement, and economic attainment over the life course (1,3,28). Despite a modest decrease in the global prevalence since 1990, an estimated 165 million children under five years old were stunted in 2011 (1), representing almost one-third of children in this age group in low- and middle-income countries (LMICs), hindering the developmental potential and human capital of entire societies.

An estimated 20% of linear growth faltering occurs *in utero* (50). Although there is wide variation between countries, 11-16% of newborns in LMICs weigh <2,500g (low birthweight [LBW]), and 27% are below the 10^{th} percentile in weight for infant sex and gestational age (small-for-gestational-age [SGA]) (165,166). At birth, the average HAZ in LMICs is -0.5 using the World Health Organization (WHO) growth standard (2,7). Infants born small show an increased risk of stunting during infancy (50–59), and subsequent growth is closely associated with prior

growth (44,45). Interventions to prevent stunting are therefore focused early in the life-course, during the critical time window from conception through a child's second birthday (the so-called first thousand days, www.thousanddays.org) (6,8).

Analyses of cross-sectional child growth data from 54 countries show that the average HAZ at birth in LMICs is below zero, then progressively declines to become stunted before 24 months, with little to no recovery thereafter (7). Average linear growth patterns are similar in children followed prospectively from birth, however, trajectories vary across cohorts (81,167–169). Aggregating data across countries may also obscure differences in growth patterns between individuals versus within a population. For example, a study of institutionalized children below two years old in Portugal identified four patterns of linear growth during nine months of follow-up (114). Children may also catch-up from growth faltering during diarrhea-free periods (82–85). In the 2-3 years after birth, a large proportion of infants show upward growth across major growth curve percentiles, suggesting catch-up (44,170,171). However, equal proportions of infants show downward growth across percentiles (44,170,171), and catch-up growth during infancy in LMICs may be limited due to the increased frequency of illness (125) in the context of marginal diets.

The literature suggests that individual growth patterns may vary. However, the longitudinal trajectories of linear growth that arise during the first thousand days, and the factors that determine these temporal growth patterns are unclear. Available nutrition-specific interventions, predominantly targeted at the period of complementary feeding from 6-24 months, have only a modest benefit on linear growth in children younger than five (6). Countries in sub-Saharan Africa will be unlikely to achieve the ambitious global targets set for child stunting reduction if current trends in prevalence and population growth persist (4). Understanding the temporal patterns of linear growth in infants, the timing and nature of growth faltering or recovery, and the factors that determine an infant's trajectory are critical to our ability to design, implement and properly time interventions that might best promote healthy growth.

In this analysis, we characterize the linear growth trajectories of HIV-unexposed Zimbabwean infants from birth through two years of age, by clustering infants with similar longitudinal growth patterns. We then identify socio-demographic and epidemiological factors associated with each growth trajectory.

Methods

Study Population

We utilized data from Zimbabwe Vitamin A for Mothers and Babies (ZVITAMBO), a randomized, placebo-controlled trial that evaluated the effect of a single large dose of vitamin A given to postpartum women and/or their infants on breast-feeding-associated mother-to-child transmission of HIV, and HIV-free survival (172). The primary outcomes and details of this cohort have been previously described (172,173). In brief, 14,110 mother-infant pairs were enrolled at 14 maternity clinics and hospitals in Harare, Zimbabwe between 1997-2001. Study participants were recruited ≤96 hours after delivery, and were followed-up when infants were ages six weeks and three months, then 3-monthly until age 12-24 months in a dedicated study clinic. Data on maternal education, socio-demographic, anthropometric variables, and paternal education were collected at baseline. Data on infant demographic, anthropometric and clinical outcomes were collected at each study visit. Socio-demographic and clinical data were collected using interviewer administered questionnaires and through transcription of data from health facility records. Infant weight and height were measured using an electronic scale (Seca Model 727, Hanover, MD, USA), and length board (ShorrBoard, Olney, MD, USA), respectively, using methods described by Gibson (174).

A total of 9,212 infants were HIV-unexposed, meaning they were born to mothers who tested HIV-negative at baseline and remained HIV-uninfected throughout follow-up. Although the trial planned to follow mother-infant pairs through 24 months, due to budgetary constraints 3,338 mother-infant pairs had longer than 12 months of follow-up. We restricted our analyses to this

subset of 3,338 participants in order to investigate linear growth beyond infancy in an HIV-unexposed infant population.

Definition of Variables

Growth was expressed as HAZ or weight-for-height z-scores (WHZ) using the WHO growth standard (2) with WHO Anthro version 3.0.1 (http://www.who.int/childgrowth/en). Gestational age was estimated according to Capurro *et al.* (175). Breastfeeding was categorized as exclusive, predominant or mixed up to age three months, as previously defined (176). Clinic visits and hospitalizations were defined as the total number of clinic visits or hospital admissions during the period since the previous visit, up to and including the current visit.

Clustering of Longitudinal Height-for-Age Growth Trajectories

We used *k*-means clustering for longitudinal data (KML) (177) to identify groups of infants with similar longitudinal patterns of linear growth. KML partitions a dataset into a predetermined number of groups, *k*, by grouping individuals who are most similar in their growth curves together. We used the Euclidean distance adjusted for temporal correlations as the measure of growth curve similarity, to account for both similarities in HAZ values and temporal behaviour (178). Since we had no *a priori* knowledge of the exact number of distinct trajectory groups in this cohort, we searched for two to nine groups using random seed selection to set the starting conditions. Since cluster results may depend on initial seed selection, we re-ran the KML algorithm 1,000 times, generating 1,000 sets of trajectory groups each, for *k*=2 to 9. To choose the optimal number of clusters and best cluster result, the Calinski-Harabatz criterion was used as the measure of cluster quality (179). This criterion is the ratio of between-cluster to within-cluster variances. A larger criterion value indicates greater separation between groups and greater homogeneity within groups, thereby indicating better clustering. The optimal cluster number and best cluster result from the 1,000 runs were chosen as the data partition that maximized the Calinksi-Harabatz criterion.

Multiple Imputation of Missing Longitudinal Data

Due to the planned study curtailment, losses to follow-up and incomplete measurement of anthropometry, there was a substantial amount of missing height/length data overall (*Table 1*). Approximately 30% of infants were missing a length measurement at any visit from birth to nine months, while up to 65% were missing a length measurement from 12-24 months. To address this we applied a data imputation strategy using multiple imputation by chained equations (MICE) (180). We used predictive mean matching (181), with all available baseline variables (presented in *Table 1*) included in the imputation model, together with HAZ at the previous visit, WHZ at the previous visit, breastfeeding to age three months, infant age, visit number, and total number of clinic visits and hospital admissions at each visit. WHZ is a strong predictor of HAZ, so we also imputed WHZ using the same model, since similar rates of missingness were observed for this variable (*Table 1*).

We generated 50 complete datasets by imputation and applied the KML algorithm to each as described above. We followed the framework for multiple imputation in cluster analysis proposed by Basagaña *et al*. (182) to select the final number of clusters, k_{fin}: the number of clusters, k, that was most often selected as optimal across all 50 complete datasets using the Calinski-Harbatz criterion. The complete datasets for which k_{fin} was chosen as the optimal number of clusters were retained for further analysis. To graphically present longitudinal growth in each cluster, we estimated average growth trajectories by fitting generalized additive models for HAZ against infant age in months at each visit, over all retained complete datasets, using cubic b-splines with four knots for smoothing.

Multiple Imputation of Missing Baseline Data

Rates of data missingness were very low for most baseline variables. However, maternal height, time until first breastfeeding, and time since last birth had high frequencies of missing values (*Table 1*). We opted to impute missing data for these variables as well for multinomial regression analyses. We used predictive mean matching for this purpose, with all available baseline variables included in the imputation model.

Multinomial Regression

We used multinomial regression to determine socio-demographic and epidemiological variables that explained differences in trajectory membership. The outcome was defined as a nominal variable specifying HAZ trajectory group membership. We fitted a separate model for each baseline variable (*Table 1*). We also fitted a multivariable model that included maternal education, age, mid-upper arm circumference (MUAC), and height; and infant sex, birthweight, length, and gestational age based on documented associations of these variables with linear infant growth. Model results were pooled over the retained complete datasets using the method proposed by Rubin (183).

All data analyses were performed in R version 3.1.2, using *KmL* (184), *mice* (180), and *nnet* (185) to implement the KML algorithm, MICE, and multinomial regression, respectively.

Results

Cohort Description

Baseline characteristics for 3,338 mother-infant pairs are shown in *Table 1*. Mothers were 24 years of age (95%CI: 24.0,24.3) on average at enrollment, and had an average of 2 children prior to the current pregnancy (95%CI: 1.94,2.03). The majority of infants were born at term (mean 39.3 gestational weeks; 95%CI:39.30,39.4 39), and approximately half were male (51.9%, 95%CI: 50.2, 53.6). Infant feeding to age 3 months was predominantly mixed (43.4%, 95%CI: 41.8, 45.1). Exclusive breastfeeding was least frequent (2.6%, 95%CI: 2.1, 3.1). One fifth of infants were stunted (HAZ<-2) by age 12 months, and almost a third were stunted by age 24 months (*Table 1*). Our imputed data were very similar to the available, non-missing data (*Table 1, Figure S1*).

Clustering

KML identified five as the optimal number of clusters in 18 of 50 (36%) of complete datasets (*Figure S2*). These 18 imputed datasets were retained for all further analyses. On average, all

five clusters showed a tendency to decline in linear growth during the follow-up period. However, they varied in their rate and timing of decline (*Figure 1*).

A median 17% of infants were identified as belonging to Group A. This group had the highest HAZ values at birth (*Table 2*), and showed an initial increase in HAZ until age six months, followed by a subsequent decline during the remainder of follow-up (*Figure 1*). These infants were persistently larger on average than other infants. Rates of stunting were low for the majority of follow-up, although 16.3% (95%CI: 2.0,30.7) were stunted at 24 months (*Table 2*). Groups B, C and D were most similar in their average trajectories, but were distinct in the timing and rates of decline (*Figure 2*). At birth, Group B (median number of infants: 23%) had similar average HAZ to Group A (*Table 2*), but did not show the same initial increase in HAZ. Prevalence of stunting was relatively low for the majority of follow-up and the most rapid faltering occurred after age nine months. In Group C (median number of infants: 23%) the average HAZ was lower at birth, declined more linearly compared to Groups B and D, and the prevalence of stunting was higher than in Group B by age 12 months. In Group D (median number of infants: 21%), a quarter of infants were stunted by six months, the average HAZ declined most rapidly between six and 15 months, and the prevalence of stunting was higher throughout follow-up than in B and C. However, by the end of follow-up, average HAZ and stunting prevalence in Groups B, C and D were similar (*Table 2*). Group E (median number of infants: 16%) comprised the group of infants with the greatest and earliest linear growth restriction. The average HAZ in this group was persistently lower than in the other groups, and a third of infants were stunted by age six months with a peak stunting prevalence of 66.6% (95%CI:54.7, 79.4) at 18 months (*Table 2*). Although the HAZ in this group showed an increase from 18 to 24 months of approximately 0.5 standard deviations (*Table 2*, *Figure 1*), almost one half of these infants remained stunted at the end of follow-up. Average growth trajectories when three was chosen as the optimal number of clusters (in 14 of 50 complete datasets) are presented in *Figure S3,* and descriptive statistics per group are presented in *Table S1*.

Multinomial Regression

In unadjusted models, membership in Group A relative to C was associated with mother's education, MUAC, height, and infant breastfeeding, birthweight and sex, while membership in Group E relative to C was associated with infant breastfeeding, sex, gestational age, birthweight and length. Each 100 gram increase in birthweight, 1 cm increase in maternal height, and 1 year increase in maternal education at baseline increased the odds of membership in Group A by 14% (OR: 1.14, 95%CI: 1.05, 1.24), 6% (OR: 1.06, 95%CI: 1.03, 1.10), and 12% (OR: 1.12, 95%CI: 1.02, 1.22) respectively. Male sex reduced the odds of memerbship in Group A (OR: 0.75, 95%CI: 0.57, 0.99) (*Table 3*). The odds of membership in Group E decreased by 22% (OR: 0.98, 95%CI: 0.66, 0.91) for each 1 week increase in gestational age, by 15% (OR: 0.85, 95%CI: 0.77, 0.93) for each 100 gram increase in birthweight, and by 19% (OR: 0.81, 95%CI: 0.68, 0.96) for each 1 cm increase in birth length. Male infants also had 46% (OR: 1.44, 95%CI: 1.09, 1.97) greater odds of membership in the Group E.

After adjustment for confounding, maternal education and height, birthweight, and infant sex were significantly associated with group membership (*Table 4*). Maternal height (OR: 1.06, 95%CI: 1.03, 1.10) and education at baseline (OR: 1.10, 95%CI: 1.01, 1.21), and birthweight (OR: 1.18, 95%CI: 1.09, 1.27) were associated with increased odds of membership in Group A. While Group E membership was associated with male sex (OR: 1.97, 95%CI: 1.32, 2.94) and birthweight (OR**:** 0.87, 95%CI: 0.79, 0.94). Some evidence for reduced odds of membership in Group E was also observed for maternal height (OR: 0.97, 95%CI: 0.94, 1.00). No baseline covariates significantly explained the probability of membership in Groups B and D relative to Group C. Multinomial regression results for group membership with three chosen as the optimal number of clusters did not change the results (*Table S2*). The same four antenatal factors explained group membership in the best and worst growing groups.

Discussion

Analyses of cross-sectional survey data from 54 countries have shown that on average infants in LMICs are born below the WHO standard for length, and decline rapidly to a z-score of

approximately -1.75 by age 24 months (7). These declines are more pronounced in sub-Saharan Africa and Asia, leading to a huge burden of stunting in these regions. In this representative birth cohort of HIV-unexposed mother-infant pairs recruited from 14 maternity clinics in Harare, Zimbabwe, we observed an overall tendency for linear faltering that is in agreement with these early and rapid declines in average HAZ. However, we identified five distinct trajectory categories into which infants can be grouped from birth to their second birthday. Although these groups were all characterised by worsening linear growth faltering during the follow-up period, the timing and steepness of growth declines varied. Infant membership in the two most extreme groups (A and E) was predicted by similar maternal and infant baseline characteristics, although the relationships were in opposite directions.

Only one other publication has examined longitudinal linear growth trajectories in infants as the unit of analysis (114). This study, from a high-income setting (Portugal) identified four groups of infants with distinct linear growth patterns: a normal stable group, a low stable group, an improving group, and a declining group. The Portuguese study evaluated a much smaller group of institutionalized children (n=49), up to 21 months of age at enrolment, followed-up for a shorter duration (nine months), and aimed specifically to evaluate the impact of institutionalization on infant growth. Despite these differences, birthweight and length were also identified as significant predictors of group membership in the Portuguese study. Children in the persistently low group were significantly smaller in length [mean: 45.4cm vs. 49.7cm, p=0.003] and weighed less [mean: 2.59kg vs 3.49kg, p=<0.001] at birth compared to the persistently high group.

While we did not investigate stunting (HAZ<-2) as an outcome, our findings are consistent with the literature on risk factors for stunting (36,51–55,57–59,75,76). Analyses of longitudinal data from 19 LMIC cohorts found 2.90 times greater odds (95%CI: 2.56, 3.33) of stunting between 12 and 60 months of age in infants born LBW compared to normal weight infants (50). Multi-country analyses of household survey data found a reduction in the risk of stunting (RR=0.968; 95%CI: 0.967-0.968) in children younger than five years for each 1 cm increase in maternal

height (75); and analyses of data from five LMIC birth cohorts indicated a 0.037 (95%CI: 0.033,0.040) increase in HAZ at age two years for every 1 cm increase in maternal height (36).

Newborn weight reflects the biological role of the intrauterine environment on fetal growth and development (8), and the overall adequacy of nutrient intake during pregnancy to meet maternal and fetal needs (65). Micro- and macronutrient supplementation during pregnancy are important determinants of birth size (68–72), and may be important determinants of infant growth (6,73,74). Short maternal stature also poses constraints on fetal growth (36,75) and reflects poor nutrition during the mother's own growth and development, which may impair maturation of organ systems and reduce a mother's capacity to deliver nutrients to her fetus when she reaches reproductive age. Maternal height may further reflect genetic effects and growth potential (36), and may be a proxy for socioeconomic status and access to adequate foods (75). Maternal education is another proxy for socioeconomic status, and determines maternal behaviours and practices. Maternal nutrition during and prior to pregnancy (67), education, and other determinants of intrauterine growth may be important areas for early intervention to promote healthy infant growth and interrupt the intergenerational cycle of stunting.

However, our results suggest that even infants with adequate birthweight, who were born to taller mothers and showed an early tendency toward healthy growth, soon suffered from linear faltering despite persistently larger attained growth than other infants. Other groups experienced high rates of stunting before age six months (Groups D and E), or tended to experience much later stunting and to maintain an average HAZ within the mild stunting range (-2≤HAZ<-1) (Groups B and C), although mild stunting is also associated with an increased risk of mortality (186). Current interventions that target stunting focus on the age of complementary feeding between 6-24 months, and even then have limited efficacy (6). The most appropriate interventions for infants may vary depending on the pattern of linear growth.

In addition to exclusive breastfeeding (EBF) from birth to six months, infants may also benefit from probiotic and prebiotic supplementation, which have been found to be safe and to improve weight gain in early infancy (20,21). Recurrent infections and diarrhea are other factors associated with linear growth faltering (1,77–82,84,85). Environmental enteric dysfunction (EED), a sub-clinical disorder of small intestinal inflammation and permeability that is common among infants living in conditions of poverty, is also associated with poor linear growth in infancy (87,88). Water, sanitaton and hygiene (WASH) interventions may be effective at reducing pathogen exposure and related linear deficits as shown in RCTs (22). The impact of WASH interventions implemented during pregnancy, in combination with nutritional supplementation from age 6-24 months is currently being investigated (187–189). The high burden of often asymptomatic enteric infections in infants in LMICs (190,191) suggests that careful consideration of the benefits and potential risks (192–194) of antibiotics as an adjunct to other interventions, including promotion of EBF (192) and complementary feeding (159), may be warranted.

A number of studies have found male infants to be more susceptible to malnutrition than female infants (51,53,58,125,195). In Zimbabwe the prevalence of stunting in children under five years in 2010-11 was 35.7% among boys and 28.3% among girls (196). This finding is typical of most countries for which data are available (195,197). Overall, there is a substantial body of evidence that boys show higher rates of clinical illness in early life and experience higher mortality (198–200). The biological reasons for the difference are unknown, but may be related to differences in inflammatory and immune responses between males and females as suggested by sex differences in immune response to vaccination (201) and infectious diseases (200). It has also been suggested that natural selection may favor maximizing reproductive fitness, resulting in higher morbidity and mortality rates in male infants to compensate for greater rates of male births (202,203).

Strengths and Limitations

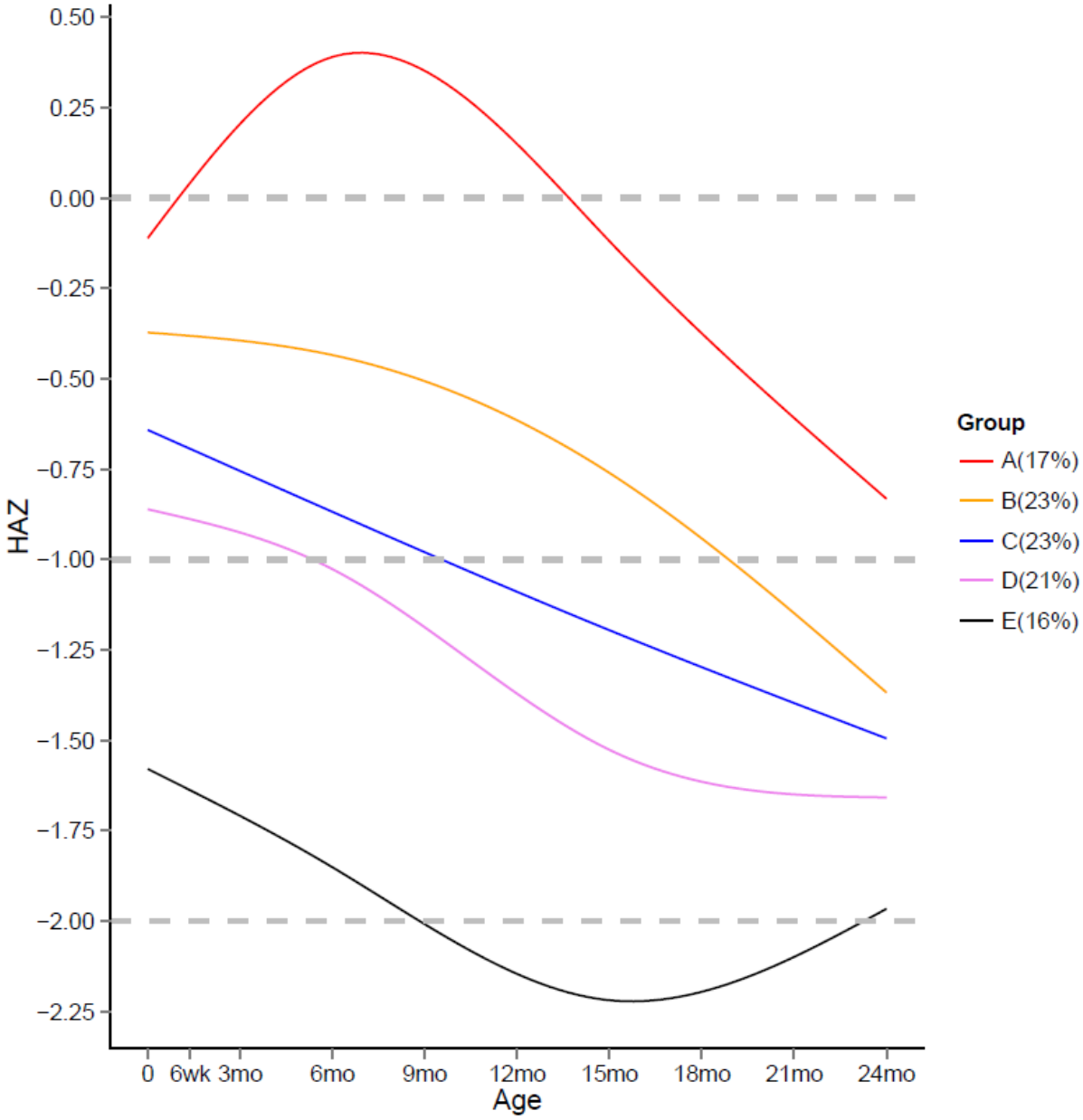
Determining the "correct" number of clusters is fundamental to cluster analysis, however there is currently no optimal method to validate the choice of cluster number (177). We utilized the Calinski-Harabatz criterion which has been shown to outperform other common measures of cluster quality on simulated data (204,205). It is also robust to a number of factors that may affect final cluster number selection (206). In addition, cluster analysis does not assign individuals to a cluster with 100% certainty. However, utilizing a multiple imputation framework allows for some of the uncertainty in cluster membership assignment to be accounted for in the analyses (182). Applying multiple imputations also allowed us to retain all available subjects in our analyses and avoid selection bias due to differential attrition, and imputed data were very similar to non-missing data at each follow-up visit. The trajectory groups we identified may not be generalizable to infants outside of this cohort, and analyses of birth cohorts in other LMICs are needed to confirm these longitudinal growth patterns. Other key variables, such as access to clean water and sanitation were not available for us to assess as determinants of trajectory membership. Baseline covariates could only explain membership in the two most extreme groups. Larger samples may be needed with greater power to detect baseline differences between the most similar trajectory groups.

Conclusion

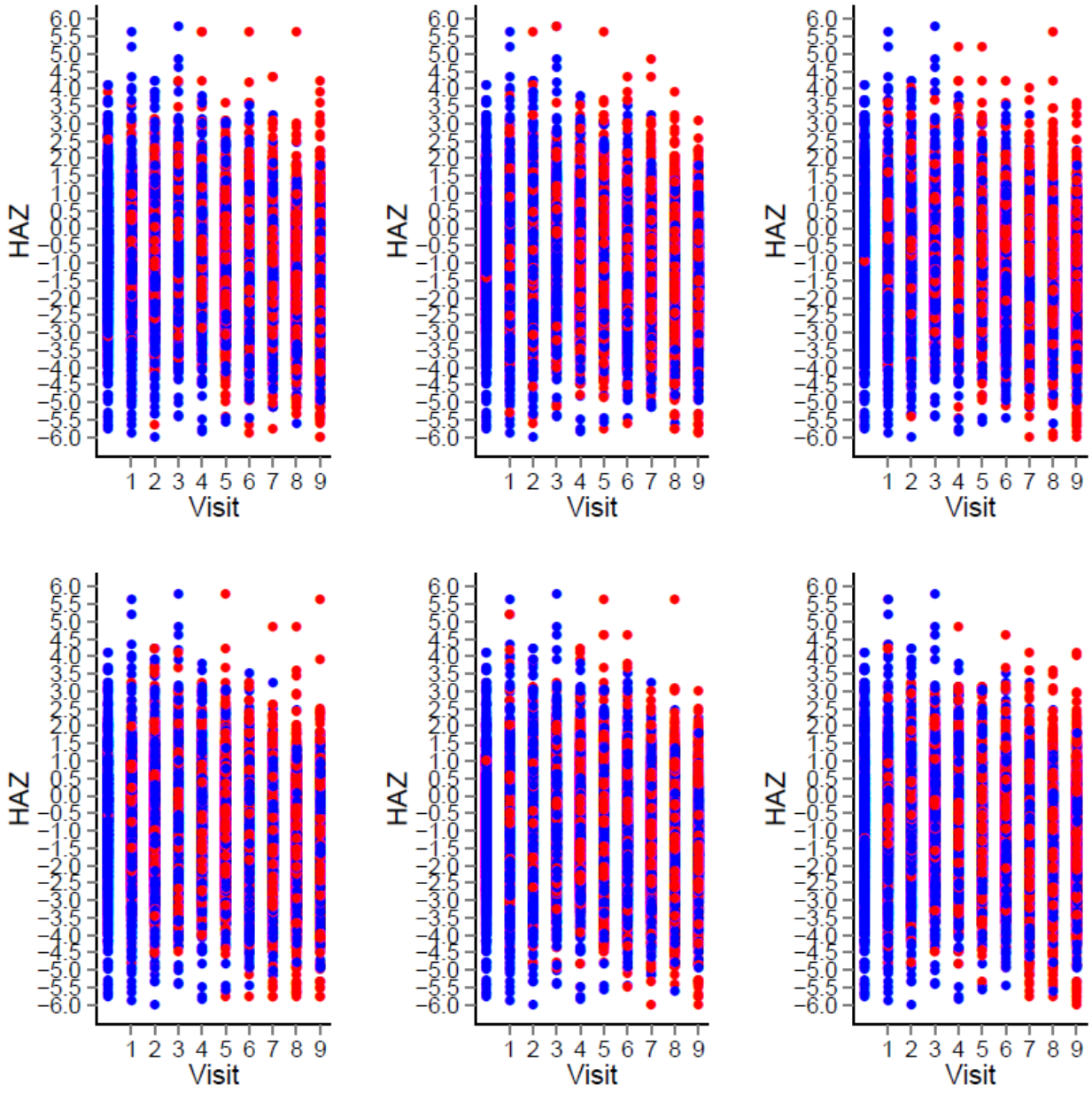
We performed our analyses on a large, representative birth cohort from Zimbabwe, followed from birth through two years of age, and found five groups of children with distinct patterns of linear growth. The nutritional status and reproductive capacity of the mother, under environmental conditions common to LMICs, and intrauterine growth may play important roles in determining individual growth curves. However, the literature has shown that factors which arise during infancy are also important. The most appropriate interventions for each group of infants may differ. More age-targeted interventions may be required during this critical period of early life.

10-1. Figure 1. Average Linear Growth Trajectories from Birth to 24 Months of Age in the Five Identified Trajectory Groups, Smoothed Across 18 Complete Datasets.

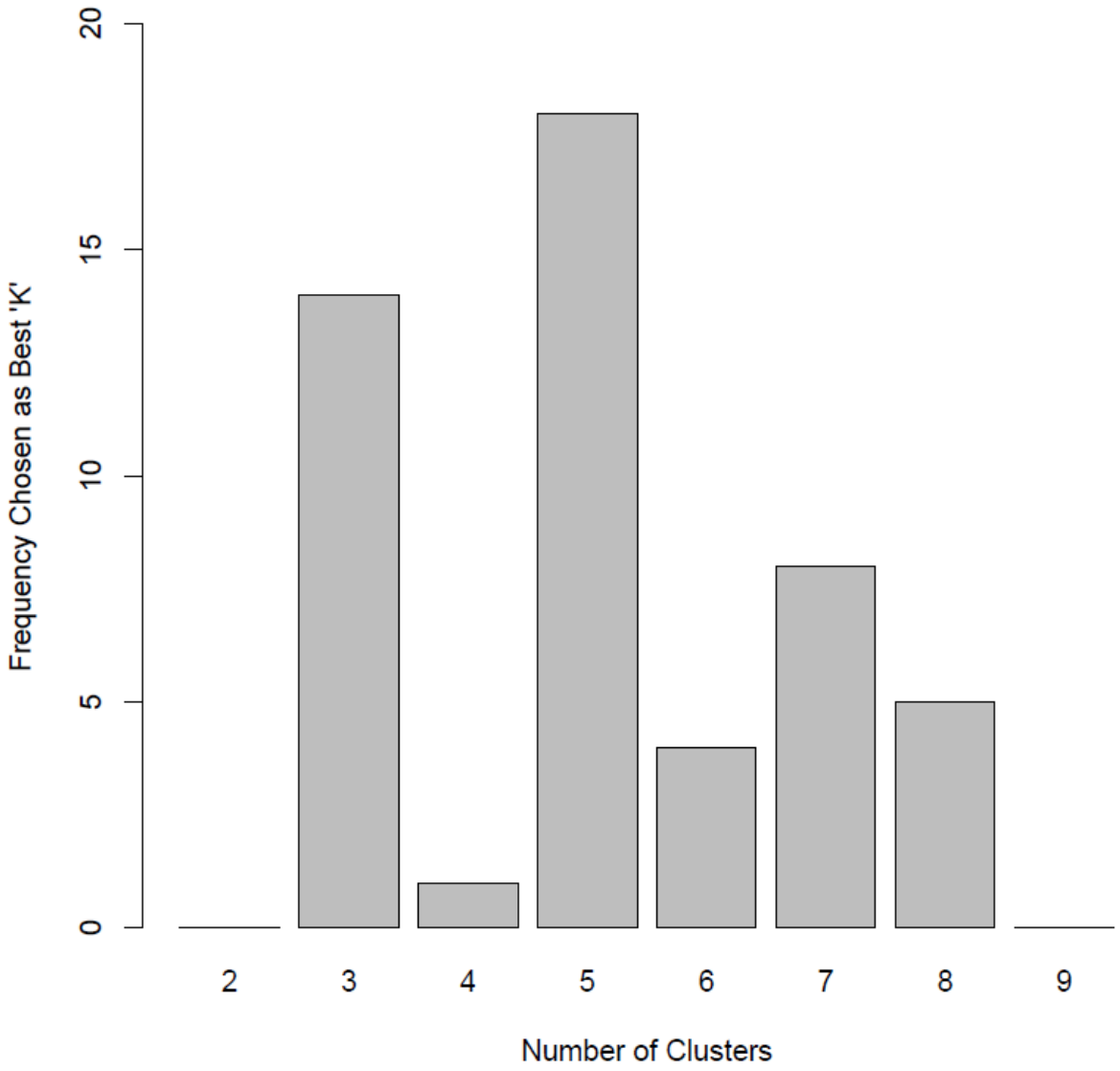
Dashed lines indicated HAZ cut-offs for the WHO reference standard median (HAZ=0), mild stunting (HAZ<-1) and stunting (HAZ<-2). Median (min-max) proportion of infants in each group were: A: 17% (15%-20%); B: 23% (22%-25%); C: 23% (21%-24%); D: 21% (19%-24%); E: 16% (14%-18%).



10-2. Supplemental Figure S1. Scatterplots of Observed (Blue) and Imputed (Red) HAZ at each Follow-up Visit in Six Randomly Selected Complete Datasets.

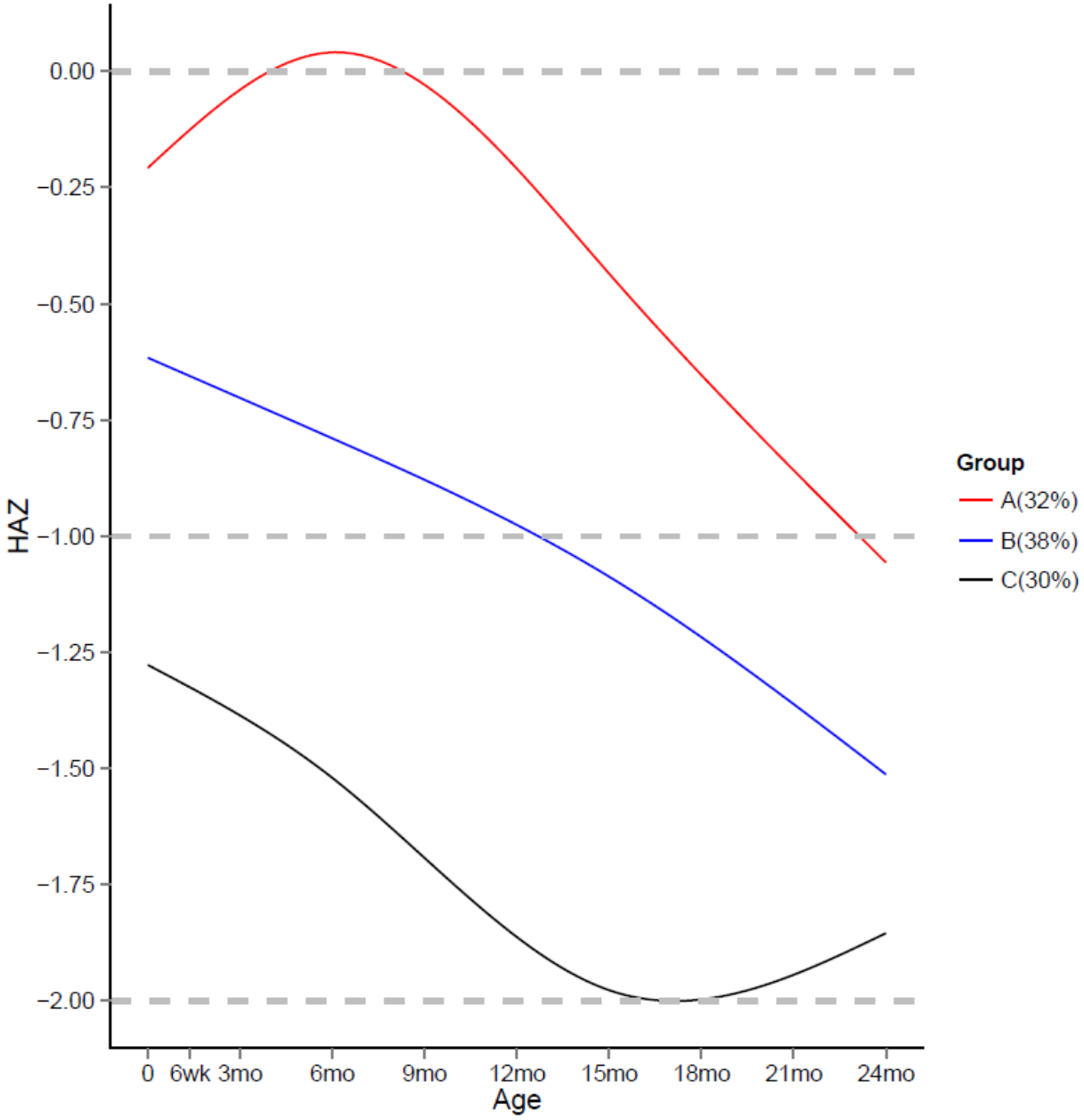


10-3 Supplemental Figure S2. Number of Complete Datasets where each Cluster Number was Chosen as Optimal.



10-4 Supplemental Figure S3. Average Linear Growth Trajectories from Birth to 24 Months of Age in the Three Identified Trajectory Groups, Smoothed Across 14 Complete Datasets.

Dashed lines indicated HAZ cut-offs for the WHO reference standard median (HAZ=0), mild stunting (HAZ<-1) and stunting (HAZ<-2). Median (min-max) proportion of infants in each group were: A: 32% (30%-34%); B: 38% (36%-39%); C: 30% (28%-32%).



10-1. Table 1. Description of Cohort (n=3,338).

	Missing (%)	Observed Mean(95%CI)	Imputed[1] Mean(95%CI)
Maternal Age (years)	0.2	24.2(24.0,24.3)	24.2(24.0,24.4)
Maternal Education (years)	0.1	9.73(9.67,9.80)	9.73(9.67,9.8)
Maternal Height (cm)	30.5	160.1(159.8,160.4)	160.1(159.9,160.4)
Maternal MUAC (centimeters)	0.6	25.9(25.8,26.0)	25.9(25.8,26.0)
Cesarean Section (%)	1.0	8.5(7.5,9.5)	8.5(7.5,9.4)
Time since Last Birth (years)	50.2	4.34(4.23,4.45)	4.04(3.95,4.14)
Number of Prior Children	0.0	2.0(1.9,2.0)	2.0(1.9,2.0)
Paternal Education (years)	2.2	10.65(10.59,10.70)	10.64(10.58,10.69)
Infant Sex (% Male)	0.1	51.9(50.2,53.6)	51.2(50.2,53.6)
Gestational Age (weeks)	0.8	39.34(39.30,39.39)	39.34(39.3,39.39)
Preterm (% <37 weeks)	0.8	6.3(5.6,7.1)	6.3(4.5,7.2)
Birth Length (centimeters)	1.1	48.4(48.3,48.5)	48.4(48.3,48.5)
Birthweight (grams)	0.3	2995(2980,3010)	2996.3(2981,3011)
Low Birthweight (% <2,500 grams)	0.3	12.4(11.3,13.6)	12.4(11.3,13.5)
Time Until First Breastfeeding (hours)	11.5	2.8(2.6,3.0)	2.8(2.6,3.0)
Breastfeeding (%)			
Exclusive	0.0	2.6(2.1,3.1)	2.6(2.1,3.1)
Partial	0.0	15.3(14.1,16.5)	15.3(14.1,16.5)
Mixed	0.0	43.4(41.8,45.1)	43.4(41.8,45.1)
WHZ			
Birth	34.2	-0.40(-0.46,-0.35)	-0.34(-0.40,-0.29)
6 months	40.8	0.43(0.36,0.48)	0.42(0.37,0.47)
12 months	52.2	0.08(0.02,0.13)	0.15(0.09,0.20)
18 months	63.5	-0.01(-0.06,0.04)	0.04(0.00,0.09)
24 months	76.5	0.02(-0.05,0.08)	-0.06(-0.12,0.00)
Number of Clinic Visits/Infant			
Birth to 6 months	0.0	1.14(1.10,1.17)	1.14(1.10,1.17)
6 to 12 months	0.0	0.81(0.78,0.84)	0.81(0.78,0.84)
12 to 18 months	0.0	0.60(0.57,0.62)	0.60(0.57,0.62)
18 to 24 months	0.0	0.31(0.29,0.33)	0.31(0.29,0.33)
Number of Hospital Admissions/Infant			
Birth to 6 months	0.0	0.047(0.039,0.054)	0.047(0.039,0.054)
6 to 12 months	0.0	0.022(0.017,0.027)	0.022(0.017,0.027)
12 to 18 months	0.0	0.017(0.012,0.021)	0.017(0.012,0.021)
18 to 24 months	0.0	0.012(0.008,0.016)	0.012(0.008,0.016)
HAZ			
Birth	1.7	-0.62(-0.66,-0.58)	-0.62(-0.66,-0.58)
6 months	27.1	-0.75(-0.79,-0.70)	-0.74(-0.79,-0.69)

	12 months	45.5	-1.03(-1.09,-0.98)	-0.99(-1.03,-0.94)
	18 months	50.5	-1.33(-1.39,-1.28)	-1.28(-1.33,-1.24)
	24 months	65.3	-1.42(-1.49,-1.36)	-1.44(-1.50,-1.39)
Stunted[2] (%)				
	Birth	1.7	11.8(10.7,12.9)	11.8(10.7,12.9)
	6 months	27.1	14.0(12.6,15.4)	14.0(12.6,15.3)
	12 months	45.5	19.0(17.2,20.8)	19.0(17.5,20.4)
	18 months	50.5	26.8(24.7,28.9)	26.3(24.5,28.1)
	24 months	65.3	29.3(26.7,31.9)	31.4(29.2,33.6)

MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score; HAZ, height-for-age z-score.

[1]The 18 imputed datasets for wchih five was the optimal number of clusters.

[2]Height-for-age z-score < -2.

10-2. Table 2. Trajectory Group Descriptions.

	A Mean(95%CI)	B Mean(95%CI)	C Mean(95%CI)	D Mean(95%CI)	E Mean(95%CI)
Maternal Age (years)	24.3(23.7,24.8)	24.2(23.7,24.7)	24.2(23.7,24.7)	24.1(23.6,24.5)	24.1(23.5,24.7)
Maternal Education (years)	10.04(9.87,10.22)	9.81(9.65,9.98)	9.70(9.51,9.89)	9.63(9.40,9.87)	9.44(9.24,9.65)
Maternal Height (cm)	162.3(161.6,163.0)	160.6(159.8,161.2)	159.7(158.8,160.7)	159.4(158.6,160.2)	158.5(157.9,159.2)
Maternal MUAC (centimeters)	26.3(26.0,26.7)	26.0(25.8,26.3)	25.9(25.5,26.2)	25.8(25.4,26.1)	25.6(25.3,26.0)
Cesarean Section (%)	8.3(5.2,11.4)	8.5(6.04,11.0)	8.8(6.2,11.4)	7.7(5.02,10.5)	9.1(6.0,12.2)
Time since Last Birth (years)	4.04(3.74,4.33)	4.08(3.83,4.34)	4.04(3.82,4.27)	4.05(3.81,4.28)	3.98(3.67,4.29)
Number of Prior Children	1.9(1.8,2.0)	1.8(1.8,2.1)	2.0(1.9,2.1)	2.0(1.9,2.1)	2.1(1.9,2.2)
Paternal Education (years)	10.78(10.63,10.92)	10.68(10.53,10.83)	10.64(10.5,10.77)	10.57(10.39,10.76)	10.51(10.35,10.66)
Infant Sex (% Male)	44.6(39.7,49.5)	49.1(44.2,54.0)	51.7(47.0,56.3)	54.3(49.3,59.2)	61.0(55.6,66.3)
Preterm (% <37 weeks)	3.5(1.8,5.2)	3.9(1.6,6.3)	5.3(1.8,8.9)	7.3(4.0,10.5)	13.0(9.10,16.9)
Gestational Age (weeks)	39.53(39.42,39.64)	39.49(39.32,39.67)	39.42(39.2,39.65)	39.24(39.03,39.45)	38.93(38.75,39.1)
Birth Length (centimeters)	48.9(48.6,49.2)	49.0(48.3,49.8)	48.7(48.0,49.4)	47.7(46.9,48.4)	47.4(46.9,47.9)
Low Birthweight (% <2,500 grams)	3.3(1.1,5.5)	6.8(3.5,10.2)	10.7(3.8,17.6)	13.8(7.1,20.5)	30.9(25.2,36.6)
Birthweight (grams)	3216(3168,3263)	3086(3003,3169)	2998(2869,3127)	2931(2826,3037)	2710(2653,2766)
Time Until 1st Breastfeeding (hours)	2.5(2.0,3.0)	2.8(2.3,3.3)	2.8(2.3,3.4)	2.6(2.0,3.2)	3.1(2.4,3.8)
Breastfeeding (%)					
Exclusive	3.2(1.5,4.8)	2.6(1.1,4.0)	2.3(1.1,3.6)	2.0(0.6,3.4)	3.3(1.8,5.1)
Partial	16.8(12.9,20.8)	13.8(10.8,16.8)	13.9(11.0,16.7)	14.6(11.1,18.1)	18.8(14.5,23.1)
Mixed	47.6(42.2,53.1)	40.8(36.3,45.4)	40.2(35.5,44.8)	40.7(35.8,45.6)	50.8(45.2,56.3)
WHZ					
Birth	0.02(-0.18,0.21)	-0.41(-0.63,-0.19)	-0.50(-0.7,-0.31)	-0.13(-0.59,0.32)	-0.65(-0.99,-0.32)
6 months	0.70(0.55,0.85)	0.41(0.18,0.63)	0.31(0.06,0.55)	0.50(0.25,0.75)	0.20(0.04,0.37)
12 months	0.48(0.34,0.62)	0.27(0.10,0.44)	0.13(-0.05,0.31)	0.00(-0.17,0.16)	-0.17(-0.30,-0.04)

18 months	0.38(0.20,0.56)	0.14(-0.02,0.31)	0.06(-0.08,0.21)	-0.08(-0.26,0.09)	-0.32(-0.50,-0.14)
24 months	0.17(-0.02,0.36)	-0.01(-0.19,0.17)	-0.05(-0.24,0.13)	-0.15(-0.28,-0.01)	-0.26(-0.44,-0.08)
Number of Clinic Visits/ Infant					
Birth to 6 months	1.31(1.05,1.56)	1.06(0.91,1.22)	1.03(0.89,1.18)	1.09(0.93,1.26)	1.27(0.96,1.58)
6 to 12 months	0.87(0.67,1.07)	0.73(0.59,0.86)	0.74(0.60,0.87)	0.81(0.67,0.94)	0.98(0.71,1.25)
12 to 18 months	0.65(0.51,0.79)	0.51(0.42,0.61)	0.53(0.42,0.65)	0.63(0.51,0.75)	0.71(0.49,0.92)
18 to 24 months	0.39(0.30,0.47)	0.24(0.15,0.33)	0.26(0.16,0.36)	0.34(0.24,0.43)	0.38(0.26,0.50)
Number of Hospital Admissions/Infant					
Birth to 6 months	0.05(0.03,0.07)	0.04(0.02,0.06)	0.04(0.02,0.06)	0.04(0.02,0.07)	0.06(0.03,0.08)
6 to 12 months	0.01(0.00,0.02)	0.02(0.01,0.03)	0.02(0.01,0.03)	0.02(0.01,0.04)	0.03(0.01,0.05)
12 to 18 months	0.01(0.00,0.03)	0.01(0.00,0.02)	0.02(0.00,0.03)	0.02(0.01,0.03)	0.02(0.00,0.03)
18 to 24 months	0.01(0.00,0.02)	0.01(0.00,0.01)	0.01(0.00,0.02)	0.01(0.00,0.02)	0.02(0.01,0.03)
HAZ					
Birth	-0.28(-0.42,-0.14)	-0.27(-0.66,0.11)	-0.46(-0.86,-0.06)	-1.00(-1.40,-0.61)	-1.22(-1.47,-0.97)
6 months	0.23(-0.06,0.53)	-0.28(-0.84,0.27)	-0.65(-1.32,0.02)	-1.38(-1.84,-0.92)	-1.77(-2.00,-1.54)
12 months	0.05(-0.2,0.31)	-0.70(-1.11,-0.29)	-1.03(-1.64,-0.42)	-1.28(-1.78,-0.78)	-2.07(-2.34,-1.81)
18 months	-0.17(-0.40,0.05)	-0.89(-1.36,-0.41)	-1.43(-1.98,-0.87)	-1.65(-2.04,-1.26)	-2.38(-2.67,-2.10)
24 months	-0.88(-1.41,-0.36)	-1.39(-2.44,-0.33)	-1.52(-2.47,-0.58)	-1.58(-2.03,-1.13)	-1.88(-2.37,-1.39)
Stunted[1] (%)					
Birth	5.2(2.9,7.5)	5.7(0.4,11.1)	8.9(2.1,15.7)	17.6(10.0,25.2)	24.5(18.0,30.9)
6 months	1.2(0.0,2.9)	3.5(0.0,11.4)	8.2(0.0,19.91)	23.8(11.3,36.4)	39.5(30.4,47.7)
12 months	2.4(0.0,5.5)	8.7(1.1,16.4)	15.1(3.7,26.5)	21.2(7.1,35.3)	54.2(44.2,64.2)
18 months	2.4(0.0,5.3)	11.8(0.9,22.7)	25.7(7.6,44.1)	32.5(18.4,46.5)	66.6(54.1,79.4)
24 months	16.3(2.0,30.7)	28.4(0.0,60.0)	32.2(2.1,62.3)	34.4(19.3,49.6)	48.0(30.2,65.8)

MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score; HAZ, height-for-age z-score.
[1]Height-for-age z-score < -2.

10-3. Table 3. Unadjusted Associations between Trajectory Group Membership and Maternal and Infant Characteristics at Baseline.

	Odds Ratio(95%CI)			
	Trajectory Group[2]			
	A	B	D	E
Vitamin A Treatment Arm[1]				
AA vs PP	1.12(0.75,1.67)	1.05(0.69,1.60)	1.08(0.71,1.63)	0.96(0.60,1.55)
AP vs PP	1.04(0.71,1.53)	0.97(0.68,1.39)	1.01(0.69,1.47)	1.04(0.72,1.50)
PA vs PP	1.05(0.73,1.51)	0.98(0.67,1.43)	1.00(0.68,1.48)	0.91(0.63,1.30)
Maternal Education (years)	1.12(1.02,1.22)	1.03(0.96,1.10)	0.98(0.89,1.08)	0.94(0.88,1.01)
Paternal Education (years)	1.07(0.98,1.17)	1.02(0.92,1.12)	0.98(0.88,1.09)	0.95(0.88,1.03)
Maternal Age (years)	1.00(0.98,1.03)	1.00(0.97,1.02)	1.00(0.97,1.02)	1.00(0.97,1.03)
Maternal MUAC (centimeters)	1.05(1.00,1.09)	1.02(0.96,1.07)	0.99(0.93,1.05)	0.97(0.92,1.03)
Maternal Height (centimeters)	1.06(1.03,1.10)	1.02(0.99,1.05)	0.99(0.96,1.03)	0.97(0.94,1.00)
Time since Last Birth (years)	1.00(0.92,1.08)	1.01(0.92,1.11)	1.00(0.93,1.08)	0.98(0.89,1.08)
Parity	0.94(0.85,1.04)	0.98(0.89,1.08)	0.99(0.90,1.09)	1.03(0.93,1.14)
Cesarean Section (yes vs. no)	0.94(0.54,1.65)	0.97(0.60,1.56)	0.87(0.52,1.46)	1.04(0.62,1.75)
Infant Sex (Male vs. Female)	0.75(0.57,0.99)	0.90(0.67,1.21)	1.11(0.82,1.50)	1.46(1.09,1.97)
Gestational Age (weeks)	1.06(0.92,1.23)	1.04(0.85,1.28)	0.91(0.75,1.09)	0.78(0.66,0.91)
Birth Length (centimeters)	1.04(0.89,1.21)	1.06(0.82,1.37)	0.84(0.68,1.03)	0.81(0.68,0.96)
Birthweight (grams)[3]	1.14(1.05,1.24)	1.05(0.94,1.19)	0.96(0.85,1.09)	0.85(0.77,0.93)
Time Until First Breastfeeding (hours)	0.99(0.95,1.02)	1.00(0.97,1.03)	0.99(0.96,1.02)	1.01(0.98,1.04)

A, Vitamin A; P, Placebo, MUAC, mid-upper arm circumference.
[1]Mother-infant randomized vitamin A treatment.
[2]Group C is the referent.
[3]Odds Ratio per 100 gram change in exposure.

10-4. Table 4. Full Multinomial Regression Model of Trajectory Group Membership and Maternal and Infant Characteristics at Baseline.

	Odds Ratio(95%CI)			
	Trajectory Group[1]			
	A	B	D	E
Maternal Education (years)	1.10(1.01,1.21)	1.03(0.96,1.10)	0.98(0.89,1.08)	0.94(0.87,1.01)
Maternal Age (years)	0.99(0.96,1.02)	0.99(0.97,1.02)	1.00(0.97,1.03)	1.01(0.97,1.04)
Maternal MUAC (centimeters)	1.02(0.97,1.07)	1.00(0.96,1.05)	0.99(0.94,1.05)	1.00(0.94,1.06)
Maternal Height (centimeters)	1.06(1.03,1.10)	1.02(0.99,1.05)	0.99(0.96,1.03)	0.97(0.94,1.00)
Infant Sex (Male vs. Female)	0.61(0.41,0.90)	0.82(0.52,1.28)	1.25(0.79,1.97)	1.97(1.32,2.94)
Gestational Age (weeks)	0.94(0.83,1.06)	0.98(0.86,1.13)	0.96(0.86,1.08)	0.97(0.86,1.09)
Birthweight (grams)[2]	1.18(1.09,1.27)	1.05(0.96,1.15)	1.00(0.89,1.13)	0.87(0.79,0.94)
Birth Length (centimeters)	0.93(0.80,1.08)	1.03(0.82,1.28)	0.84(0.69,1.03)	0.87(0.75,1.01)

MUAC, mid-upper arm circumference.
[1]Group C is the referent.
[2]Odds Ratio per 100 gram change in exposure.

10-5. Table S1. Trajectory Group Descriptions with Three as the Optimal Number of Clusters.

	A Mean(95%CI)	B Mean(95%CI)	C Mean(95%CI)
Maternal Age (years)	24.3(23.9,24.6)	24.1(23.7,24.5)	24.1(23.7,24.5)
Maternal Education (years)	10.0(9.8,10.1)	9.7(9.6,9.8)	9.5(9.4,9.7)
Maternal Height (centimeters)	161.6(161.0,162.1)	160.0(159.4,160.5)	158.8(158.3,159.4)
Maternal MUAC (centimeters)	26.3(26.0,26.5)	25.9(25.7,26.1)	25.6(25.4,25.8)
Cesarean Section (%)	8.0(6.1,10.0)	8.6(6.6,10.6)	9.0(6.9,11.0)
Time since Last Birth (years)	4.1(3.8,4.3)	4.0(3.9,4.2)	3.98(3.81,4.15)
Number of Prior Children	1.9(1.8,2.0)	2.0(1.9,2.1)	2.0(1.9,2.1)
Paternal Education (years)	10.7(10.6,10.8)	10.6(10.5,10.7)	10.5(10.4,10.6)
Infant Sex (% Male)	46.4(42.9,49.9)	51.8(48.2,55.5)	57.9(54.3,61.6)
Preterm (% <37 weeks)	3.4(2.2,4.6)	5.6(4.2,7.1)	10.3(8.2,12.4)
Gestational Age (weeks)	39.5(39.4,39.6)	39.4(39.3,39.5)	39.1(39.0,39.2)
Birth Length (centimeters)	48.9(48.7,49.2)	48.5(48.3,48.8)	47.6(47.4,47.9)
Low Birthweight (% <2,500 grams)	4.4(2.8,5.9)	10.7(8.7,12.8)	23.2(20.1,26.4)
Birthweight (grams)	3168(3133,3196)	3003(2968,3038)	2805(2767,2842)
Time Until First Breastfeeding (hours)	2.6(2.3,2.9)	2.9(2.5,3.3)	2.9(2.5,3.3)
Breast Feeding (%)			
Exclusive	2.9(1.8,4.1)	2.4(1.4,3.4)	2.6(1.4,3.7)
Partial	15.4(12.8,18.0)	14.3(11.9,16.8)	16.4(13.7,19.1)
Mixed	44.4(41.0,47.9)	39.8(36.2,43.4)	46.9(43.3,50.6)
WHZ			
Birth	-0.15(-0.28,-0.01)	-0.38(-0.51,-0.24)	-0.51(-0.64,-0.37)
6 months	0.59(0.46,0.72)	0.38(0.25,0.50)	0.31(0.21,0.41)
12 months	0.39(0.30,0.48)	0.13(0.01,0.25)	-0.09(-0.19,0.00)
18 months	0.24(0.14,0.34)	0.11(-0.02,0.24)	-0.24(-0.35,-0.12)
24 months	0.05(-0.07,0.17)	-0.01(-0.18,0.16)	-0.24(-0.38,-0.10)
Number of Clinic Visits/Infant			
Birth to 6 months	1.20(1.10,1.3)	1.03(0.91,1.14)	1.20(1.08,1.32)
6 to 12 months	0.83(0.74,0.91)	0.72(0.63,0.81)	0.91(0.81,1.00)
12 to 18 months	0.60(0.54,0.67)	0.53(0.45,0.60)	0.68(0.60,0.75)
18 to 24 months	0.34(0.29,0.39)	0.24(0.18,0.30)	0.37(0.31,0.43)
Number of Hospital Admissions/Infant			
Birth to 6 months	0.05(0.03,0.06)	0.04(0.03,0.06)	0.05(0.03,0.07)
6 to 12 months	0.02(0.01,0.02)	0.02(0.01,0.03)	0.03(0.02,0.05)
12 to 18 months	0.01(0.00,0.02)	0.02(0.01,0.02)	0.02(0.01,0.03)
18 to 24 months	0.01(0.00,0.02)	0.01(0.00,0.01)	0.02(0.01,0.03)

HAZ				
	Birth	-0.29(-0.41,-0.17)	-0.55(-0.71,-0.40)	-1.06(-1.18,-0.94)
	6 months	-0.07(-0.22,0.08)	-0.67(-0.90,-0.44)	-1.56(-1.76,-1.36)
	12 months	-0.31(-0.50,-0.12)	-0.92(-1.10,-0.75)	-1.79(-1.95,-1.63)
	18 months	-0.43(-0.58,-0.28)	-1.39(-1.63,-1.16)	-2.07(-2.24,-1.90)
	24 months	-0.94(-1.36,-0.51)	-1.74(-2.39,-1.09)	-1.67(-2.01,-1.34)
Stunted[1] (%)				
	Birth	5.5(3.6,7.5)	10.7(8.1,13.2)	19.8(16.7,23.0)
	9 months	3.2(1.3,5.1)	9.8(5.7,13.8)	301.0(25.2,36.8)
	12 months	4.9(1.7,8.2)	13.6(9.7,17.4)	40.4(34.8,46.0)
	18 months	3.9(1.1,6.7)	25.6(18.3,32.8)	52.0(44.8,59.2)
	24 months	15.5(2.6,28.5)	39.2(18.8,59.5)	40.3(29.0,51.7)

MUAC, mid-upper arm circumference; WHZ, weight-for-height z-score; HAZ, height-for-age z-score.
[1]Height-for-age z-score < -2.

10-6. Table S2. Full Multinomial Regression Model of Trajectory Group Membership and Maternal and Infant Characteristics at Baseline with Three as the Optimal Number of Clusters.

	Odds Ratio(95%CI)	
	Trajectory Group[1]	
	A	C
Maternal Education (years)	1.07(1.00,1.15)	0.96(0.90,1.02)
Maternal Age (years)	1.00(0.97,1.02)	1.01(0.99,1.04)
Maternal MUAC (centimeters)	1.01(0.97,1.05)	0.98(0.95,1.02)
Maternal Height (centimeters)	1.04(1.01,1.06)	0.97(0.96,0.99)
Infant Sex (Male vs. Female)	0.69(0.53,0.89)	1.54(1.19,2.01)
Gestational Age (weeks)	0.99(0.90,1.09)	0.99(0.91,1.09)
Birthweight (grams)[2]	1.10(1.07,1.14)	0.90(0.87,0.94)
Birth Length (centimeters)	1.01(0.93,1.10)	0.93(0.87,0.99)

MUAC, mid-upper arm circumference.
[1]Group B is the referent.
[2]Odds Ratio per 100 gram change in exposure.

11. The Impact of Antibiotics on Growth in Children in Low and Middle-income Countries: A systematic review and meta-analysis of randomized controlled trials.

11.1. Preface to "The Impact of Antibiotics on Growth in Children in Low and Middle-income Countries: A systematic review and meta-analysis of randomized controlled trials."

More effective interventions are needed to prevent stunting malnutrition and to restore linear growth deficits in children. Antibiotics are indicated in the treatment of infections to reduce morbidity and mortality, and are recommended for routine use in the treatment of severe-acute malnutrtion (SAM) and in the prophylactic prevention of opportunistic infections in HIV-infected persons. Antibiotics are also known to induce growth gains in animal studies as well as in some human studies. Due to the profound impact that antibiotics are known to have, not only on pathogens, but on commensal gut microbes in general, it has been suggested that the growth gains associated with antibiotic use may result from alteration of the intestinal microbiota. In this manuscript, I quantified the average effect of antibiotics on growth in children in LMICs and identified sub-populations in which antibiotic growth benefits may be largest. This study was approached as a proof-of-concept exercise to examine whether a microbiota-altering therapy could conceivably produce growth gains in children. To do so, I performed a systematic review of the literature on RCTs in which antibiotics were used in prepubertal children and growth was measured as an outcome. The results highlight the need to clarify the mechanisms underlying the observed antibiotic growth-promoting effect, specifically to determine the relative contribution of the resolution of clinical and sub-clinical infections versus antibiotic therapy-induced microbiota alteration leading to improvements in nutrient and energy harvest for the host.

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11.2. *Manuscript: "The Impact of Antibiotics on Growth in Children in Low and Middle-income Countries: A systematic review and meta-analysis of randomized controlled trials."*

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ABSTRACT

Objective: To determine whether antibiotic treatment leads to improvements in growth in prepubertal children (1 month to 12 years old) in low- and middle-income countries, to determine the magnitude of growth improvements, and to identify moderators of this treatment effect.

Design: Systematic review and meta-analysis.

Data Sources: Medline, Embase, Scopus, the Cochrane Central Register of Controlled Trials, and Web of Science.

Study Selection: Randomized controlled trials conducted in a low- or middle-income country in which an orally administered antibacterial agent was allocated by randomization or minimization, and growth was measured as an outcome. Participants 1 month to 12 years old were included. Control was placebo or non-antimicrobial intervention.

Results: We pooled data from ten randomized controlled trials representing 4,316 children, across a variety of antibiotics, indications for treatment, treatment regimens, and countries. In random effects models, antibiotic use increased height by 0·04cm/month (95%CI:0·00 to 0·07) and weight by 23.8g/month (95%CI:4.3 to 43·3). Effects on height were larger in younger populations; and effects on weight were larger in African studies vs. other regions, after adjusting for age.

Conclusion: Antibiotics have a growth-promoting effect in prepubertal children in low- and middle-income countries. This effect is more pronounced for ponderal than for linear growth. The antibiotic growth-promoting effect may be mediated by treatment of clinical or sub-clinical infections, or possibly by modulation of the intestinal microbiota. Better defining the mechanisms underlying this effect will be important to inform optimal and safe approaches to achieving healthy growth in vulnerable populations.

INTRODUCTION

Undernutrition in early childhood, characterised by poor linear or ponderal growth, underlies approximately one third of all under-5-year mortality worldwide (1). Linear growth, measured as height or length is an indicator of long term nutritional status; children whose height-for-age lies more than two standard deviations below the reference population median are termed stunted. Ponderal growth, measured as body weight, is viewed as an indicator of short-term or long-term nutritional status. Children whose weight-for-age lies more than two standard deviations below the reference population median are termed underweight. Underweight and

stunting, particularly during the first 2 years of life, have short-term effects on morbidity and mortality and long-term effects on cognition, educational achievement, and adult economic productivity (3). Given the current global focus on reducing underweight and stunting to reach forthcoming global health targets (4,207), there is increasing interest in evaluating interventions to promote healthy growth in childhood (6). Primary interventions to improve child growth have largely focused on nutritional supplementation and diarrhoea prevention. However the impact of these interventions on restoring growth deficits in undernourished children is modest (77,83,208,209). Restoration of linear growth deficits is particularly challenging beyond the first two years of life (3).

The growth-promoting effects of antibiotics were first observed in animals in the 1940s. Small daily doses of broad-spectrum antibiotics have been found to improve average daily weight gain in farm animals by as much as 73% (149–157). These observations led to the hypothesis that food animals reared in conditions of poor sanitation and hygiene have impaired growth because of chronic exposure to environmental microbes and pathogens, and that antibiotic treatment may therefore improve growth (78).

In humans, an association between infections and malnutrition in children is supported in the literature (79,80). Nutrient harvesting from the diet and the inflammatory response of the gut are also modulated by the intestinal microbiota, a microbial ecosystem that is essential to human health and nutrition (9,10,126,128,129). Perturbation of this microbial community through chronic exposure to environmental microbes or pathogens may also be detrimental to child growth (78,90,93,210), and studies have shown that antibiotic use can affect its composition (16,17). Antibiotic use has also been associated with significant height and weight gains among children in some target populations (23,158,159,161). However, results have not always been consistent (23,211–214), and researchers continue to investigate the potential co-benefits of antibiotic therapy on child growth (215,216). The objective of this systematic review of randomized controlled trials was to determine whether improvements in growth are seen among prepubertal children (1 month to 12 years old) in low- and middle-income countries treated with antibiotics; to determine the magnitude of these growth effects; and to identify

treatment effect moderators. We hypothesized that antibiotics would have a positive average effect on both height and weight, and that treatment effect size would be moderated by the characteristics of antibiotic treatment, differences in study population, or trial quality.

METHODS

Search Strategy and Selection Criteria

This review is reported in accordance with the PRISMA statement (217) and recommendations for reporting meta-analyses of individual patient data (218). We searched Medline (including In-Process and Other Non-Indexed Citations) and Embase, both using Ovid, as well as Scopus and the Cochrane Central Register of Controlled Trials up to December 12th, 2013. All search strings were developed with the assistance of a professional librarian. Search strings are provided in *Appendix 1*. The review protocol is provided in *Appendix 2*.

We searched for randomized controlled trials conducted in a low- or middle-income country with participants 1 month to 12 years old allocated by randomization or minimization to antibacterial treatment, given by mouth, or control. Control interventions included placebo, an intervention with no known antimicrobial effect, or no treatment. Trials, published or unpublished, were selected if growth was measured as an outcome. Studies of anti-helminthic therapies were excluded, since systematic reviews of such trials have already been conducted (101,102). We placed no restrictions on language, year of publication, or the length of follow-up, and excluded quasi-experimental studies, observational studies, reviews, and simulations. We excluded studies of neonates (infants <1 month old) since growth patterns during the neonatal period, particularly among preterm infants, are very different from the post-neonatal period. Finally, trials were not eligible for inclusion if the condition being treated did not depend on the antimicrobial effect of antibiotic treatment (e.g. use of specific antibiotics to reduce feeding intolerance through pro-kinetic effects or to improve lung function through anti-inflammatory effects).

Two investigators (E.K.G. and S.M.A.J.) independently assessed titles and abstracts for eligible publications. If eligibility could not be determined, the full article was retrieved and the article methods were screened. We used Web of Science to search for publications that cited the included studies in an effort to find similar trials, and also hand-searched reference lists of included trials and any review articles we identified. Discrepancies were adjudicated by a third investigator (A.J.P.).

Data Abstraction and Analysis

Study quality was determined by assessing the included publications for risk of bias due to their procedures for sequence generation, allocation concealment and blinding; and due to informative censoring or selective outcome reporting using a standardized instrument adapted from the Cochrane Handbook (219). Included publications were assessed independently by two reviewers (E.K.G. and S.M.A.J.). Discrepancies were resolved by consensus.

Study authors were contacted up to three times by email (or by telephone if email was unsuccessful) to determine their interest in collaborating on this review, and to request individual patient data (IPD). When IPD could not be obtained, data were abstracted independently by the same two reviewers using a standardized pre-tested form, with discrepancies corrected by consensus. Per trial arm, we abstracted: number of participants, number lost to follow-up or excluded after randomization, mean baseline height or weight, and mean height or weight (and standard deviations) at the end of follow-up. We also abstracted p-values, confidence intervals, and standard errors of reported treatment effects. Where mean change in height or weight per unit of follow-up time were reported, the same information was retrieved. The following trial-level characteristics, which we defined *a priori* as potential moderators of treatment effect, were also abstracted: indication for treatment, country, proportion of males, mean age, antibiotic agent, dosage, frequency and duration of antibiotic therapy, concurrent interventions given, length of follow-up, and whether treatment effects were adjusted for baseline imbalances. We defined antibiotic class as bacteriostatic or bactericidal, and antibiotic spectrum as broad or narrow. Broad-spectrum antibiotics were

defined as those reported in the literature to be effective against a wide range of gram-positive and gram-negative bacteria, and narrow-spectrum as those reported in the literature to be effective against a limited range of bacteria (220–228). All abstracted trial characteristics are summarized in *Tables 1 and 2*. Risk of bias domains were treated as potential sources of heterogeneity.

Outcomes were: (i) mean height in centimeters (cm) or weight in grams (g) at the end of follow-up; or (ii) mean change in height (cm) or weight (g) per unit of follow-up time. The difference in means between treatment and control arms was the measure of treatment effect. Treatment effects and their variances were scaled as the average effect per month of follow-up. Height and weight were analysed separately. When a trial allocated participants to more than one intervention, data were abstracted from the arm allocated to receive antibiotics (229–231). When a trial allocated participants to more than one antibiotic arm, data from both arms were combined to avoid unit of analysis errors (159,232).

IPD and aggregate data (AD) were combined using a two-step approach (233). In the first step, treatment effects in each IPD trial were estimated in an intention-to-treat analysis using linear mixed models to allow for random intercepts and serial correlation. One IPD model was fit per trial, with baseline growth, age, sex, duration of follow-up, and a duration-by-treatment interaction included as covariates. In the second step, a random-effects model was used to pool intention-to-treat effect estimates (obtained from separate IPD trials in step 1) with intention-to-treat effect estimates abstracted from AD publications.

Statistical heterogeneity was assessed using the I^2 statistic (234). Heterogeneity was explored using weighted meta-regression and sub-group meta-analyses. Statistical significance was evaluated at $\alpha<0{\cdot}05$. Publication bias was assessed using Egger's test (234). Sensitivity analyses were performed in two ways: (i) to determine the robustness of meta-analysis results to the removal of studies (235), and (ii) by fitting linear mixed models restricted to the five trials for which IPD were available.

IPD trials were modelled using the *lme4* package, and all meta-analyses and meta-regression models were fit using the *metafor* package (236), both using *R* version 2·15·1.

RESULTS

Study Selection

The electronic search identified 4,600 records. An additional 24 records were identified through Web of Science and a backward search of reference lists (*Figure 1*). Of these, 190 studies were retrieved and screened for eligibility. One hundred thirty-nine studies failed to meet at least 2 selection criteria. Thirty-four additional studies were excluded for failing to meet the following single criteria: no antibiotic was allocated (N=8), an active comparator was used (N=7), the trial was not conducted in a low- or middle-income country (N=5), treatment was not randomized (N=5), growth was not measured or reported (N=4), participant age range exceeded 12 years (N=2), review articles (N=2), and the antibiotic was not administered orally (N=1). Only four were non-English language texts. These were screened with the help of an electronic translator.

Four additional trials were excluded because they reported differences in stunting or wasting prevalence (237–239), or reported growth using the Wetzel grid method (240). Of these, three authors could not be reached to request IPD or unpublished data (237,239,240), and data were no longer available from one (238). Another author was contacted successfully and provided IPD, but did not provide a data dictionary. Since this publication did not report outcomes for the antibiotic arms, this trial was excluded (241). Of these 5 otherwise eligible randomized controlled trials that were excluded because growth was not reported in the desired format, four reported no antibiotic growth benefits (237–239,241), but would only have represented 8% of the total person-time if they were included and would not have greatly influenced our findings. Another two trials were excluded because they only reported growth at baseline and the authors could not be reached (242,243).

Published data were available from five trials (229,231,232,244,245), and complete IPD datasets were obtained from five trials (158,159,161,230,246). Thus ten randomized controlled

trials were included in the meta-analysis (158,159,161,229–231,244–246). Only data from the secnidazole and placebo arms, and the metronidazole and placebo arms were included from Goto *et al.* (230) and Gupta *et al.* (231) respectively. Only data from the metronidazole and no intervention arms were included from Heikens *et al.* (229).

Study Characteristics

Of these ten trials, nine were placebo controlled, and one gave controls no treatment (229). Indication for treatment varied by trial and included malnutrition (N=4), *Giardia lamblia* infection (N=2), diarrhea with or without vomiting (N=2), environmental enteropathy (N=1), and prophylaxis in Human Immunodeficiency Virus-infected (HIV) children (N=1). The earliest included trial was published in 1953, and the most recent in 2013. Three trials gave participants in both arms a nutritional supplement (159,229,244) in addition to antibiotics or control (*Table 1*). Only three AD trials reported the number of male participants (*Table 2*). Two trials recruited hospitalized children, both reported weight only (158,245).

Eight trials reported height (159,161,229–231,244,246) and all reported weight. Four IPD trials reported height (159,161,230,246) and five reported weight (158,159,161,230,246). Together these trials included 1,699 control and 2,617 antibiotic treated subjects, followed-up for a mean±std 268±266 days, across seven countries. Mean trial age ranged from 4 to 115 months (*Table 2*). On average, trial participants were below the age-standardized reference population mean for height or weight at baseline (*Table 1*).

Risk of Bias

Only one trial (232) was evaluated to be high risk for bias overall (i.e. when all bias domains were considered together). This was based on: (i) high risk due to inadequate random sequence generation, since treatment was randomly allocated to groups of children determined by the investigators; (ii) unclear risk due to inadequate allocation concealment; and (iii) high risk due to differential attrition between treatment arms. Five trials were ranked as low risk for bias overall. These trials were low risk in all six bias domains (158,159,161,230,246). Finally, four

trials had an unclear risk for bias overall because the procedures were not fully described (229,231,244,245).

Regarding risk of bias due to attrition, the linear mixed models fit for the five IPD trials were unbiased by losses to follow-up, provided the losses were uninformative conditional on observed height and weight. Among the AD trials, only Guzman *et al.*(232) was determined to be impacted by drop-outs. Gupta *et al.*(231) reported exclusions prior to randomization, but reported outcomes on all 79 subjects recruited at baseline. Heikens *et al.*(229) reported that drop-outs predominantly consisted of participants who moved too far from the study site to be followed-up or withdrew consent (9% and 2% of the total sample respectively). Risk of bias due to attrition could not be assessed in the two remaining AD trials because they did not provide any data on participant exclusion during the study (244,245). However, these trials only accounted for 5.9% (244) of the weights in the pooled treatment effect for height, and 1.2% (244) and 1.4% (245) in the pooled treatment effect for weight. Overall, we do not think that attrition posed a serious risk of bias in our analyses.

With respect to publication bias, Egger's test suggested no significant bias among trials reporting height (p=0·841) or weight (p=0·391).

Meta-analysis

Our random effects models estimated an average treatment effect for height of 0·04cm/month (95%CI:0·00 to 0·07) (*Figure 2*), and an average effect for weight of 23.8g/month (95%CI:4.3 to 43·3) in antibiotic-treated versus control children (*Figure 3*). The I^2 statistic showed a considerable degree of statistical heterogeneity in both height and weight treatment effects (84·8% and 84.4% respectively).

In order to assess the impact of antibiotic treatment on growth in children <2 years old, we used the same two step approach described for the analysis using complete data. We fitted IPD models restricted to participants <2 years old (158,159,161,230), and pooled these treatment effect estimates with the AD estimates reported by Heikens *et al.*,(229) which was the only AD

trial restricted to this age group. These included observations from 833 control and 1,461 treated infants, followed-up for 169±152 days on average. The treatment effect in these children was not statistically significant for height (0.03cm/month; 95%CI:-0.05 to 0.11), but was for weight (29.6g/month; 95%CI: 2.4 to 56.8), I^2=47.0%.

Meta-regression Analyses

Only geographic region significantly explained variation in the treatment effect across trials for weight (*Table 3*). The treatment effect was 35.6g/month larger on average in trials conducted in Africa (95%CI:12.8 to 58.3) compared to trials conducted in other regions. No statistically significant moderators of the height treatment effect were identified by bivariate analyses. We could not investigate risk of bias domains as moderators of treatment effect because only one trial was evaluated as high risk in any domain. All bivariate models included one treatment effect moderator and one outcome (*Table 3*).

Duration of treatment, geographic region, treatment for *Giardia lamblia*, and age were statistically significant moderators of treatment effect, after adjustment for mean study population age (*Table 4*). The height treatment effect was 0.001cm/month (95%CI: -0.002 to 0.000) smaller on average with each one month increase in mean population age, and was 0.007cm/month larger on average with each additional day of treatment (95%CI: 0.00 to 0.01). The weight treatment effect was 0.5g/month smaller on average (95%CI: -1.0 to -0.1) with each one month increase in mean age, was 33.2g/month (95%CI: 5.3 to 61.2) larger on average in trials conducted in Africa, and was 46.9g/month (95%CI: -83.2 to -10.6) smaller on average in trials in which participants were treated for *Giardia lamblia* infection. In this last model, the intercept was 62.1g/month (95%CI: 29.3 to 94.9), indicating a significant treatment effect in trials that did not treat children for *Giardia lamblia*. All mean age adjusted models included mean participant age, one treatment effect moderator, and one outcome (*Table 4*).

Sensitivity Analyses

Only removal of Prendergast *et al*. (161) from the random effects model impacted the average effect for height. Without this trial, the average effect was 0·02cm/month (95%CI: -0·01 to 0·05), a 50% decrease. The average treatment effect for weight was robust to the removal of trials. Also, two trials recruited hospitalized children (158,245). Simultaneous exclusion of both trials did not change the average treatment effect for weight (21.5g/month; 95%CI: 2.3 to 40.7). These two trials did not report height.

In addition, we fit linear mixed models in order to investigate whether adjusting on participant age at the individual level (using IPD trials only) would produce the same estimates of treatment effect moderation as we obtained by weighted meta-regression adjusted on mean participant age (*Table 4*). These models included age and duration of treatment, geographic region, or treatment for *Giardia lamblia* infection, along with corresponding interaction terms. Results of these IPD models were consistent with the weighted meta-regression results using all trials, with the exception of age, where the treatment effect on weight increased by 0.8g/month for each one month increase in child age on average.

Subgroup Analyses

The weight treatment effect was homogeneous across trials conducted in Africa using a random effects model (41.4g/month; 95%CI: 31.0 to 51.7); I^2=0.0%. The average treatment effect estimated in this sub-group was identical when a fixed effects model was used (41.4g/month; 95%CI: 31.0 to 51.7).

DISCUSSION

Principal Findings

In this pooled analysis of individual patient data and aggregate data from ten randomized controlled trials conducted in seven low- and middle-income countries, antibiotic treatment had a positive average treatment effect on both height and weight in children 1 month to 12 years old. Our results suggest that the growth-promoting effect of antibiotics is more substantial for ponderal than for linear growth, and the effect may be more homogenous in

younger children. Analysis of individual data from the IPD trials showed an increase in the weight treatment effect with increasing participant age. This is in contrast to the meta-regression model results which suggested a smaller effect with increasing mean age. IPD trials primarily included children <5 years old, whereas two AD trials recruited older children. Cross-level effects may also partly explain this discrepancy. While we did not restrict study selection to populations with a particular nutritional status, children were generally below the age-standardized reference population mean for height or weight, reflecting the spectrum of stunting and wasting malnutrition seen in low- and middle-income countries. The larger weight treatment effects we observed in trials conducted in Africa may plausibly be explained by the high prevalence of HIV infection and severe acute malnutrition among populations included in these studies. Two trials conducted in Africa included severely malnourished children in whom all or a subset were HIV-infected or exposed (159,161). A third trial also included children from a similar high HIV prevalence community (159,246), although HIV status was not specifically reported. The smaller weight treatment effect we observed in trials treating children for *Giardia lamblia* suggested that growth may not be as strongly impacted in children treated with antibiotics for this specific protozoal infection. Overall, the average treatment effects we observed would correspond to an approximate 0.1 increase in height-for-age Z-score and a 0.2-0.3 increase in weight-for-age Z-score over 6 months in HIV-infected, HIV-exposed, or severely malnourished populations under 2 years old using the World Health Organization (WHO) growth standard (2). These treatment effects therefore represent clinically relevant growth gains for the youngest children from the most vulnerable populations, in whom the long-term impact of undernutrition is most profound.

Strengths and Limitations of study

The inclusion of IPD and AD trials served to improve the precision of our pooled estimates, minimized the risk of publication bias (233), and allowed us to define height and weight in uniform units, avoiding the potential sources of bias inherent in utilizing standardized mean differences (247). We synthesized data from 4,316 children, observed across a variety of antibiotics, indications for treatment, treatment regimens, and countries, providing the first

comprehensive review of evidence from all randomized trials relating antibiotic use to growth in children in low- and middle-income countries, conducted over a 60 year period. A clear limitation of pooling such a diverse set of trials, with a large degree of statistical heterogeneity, is the limited generalizability of the average treatment effects. It is not completely clear which antibiotics or treatment regimens can be expected to produce these growth effects in other populations. However, pooling this diverse set of trials did allow identification of important sub-populations in whom the growth effect may be more profound when broad-spectrum antibiotics are used. However, due to the small number of trials, we had limited power to identify moderators of treatment effect, and we were not able to fully investigate trial-level confounding with multivariable meta-regression models. Specifically, the potential modifying effect of HIV prevalence, treatment duration, antibiotic class, concurrent nutritional interventions and study population characteristics could not be fully elucidated. Also, ecological bias cannot be ruled out in our meta-regression analyses of treatment effect moderators (which are measured at trial level); hence care must be taken in extending the treatment modifying effects to the individual level, particularly for age. Egger's test showed no evidence of publication bias. Careful screening of search results and communication with investigators ensured identification of published and unpublished reports. Finally, only one trial was evaluated to be high risk for bias (232).

Comparison with other Studies

The exact reasons for the observed antibiotic growth effects remain unclear, but a number of mechanisms may be involved. Respiratory and gastrointestinal infections are known to be associated with undernutrition. Nutrient malabsorption, increased nutrient loss during episodes of diarrhea, gut inflammation, impaired intestinal barrier function, diversion of nutrients away from growth to support immune activation, and loss of appetite are possible reasons for impaired growth during infection (78–80). Antibiotics may improve growth by resolving sub-clinical and clinical infections. Eradication of microbes that regulate endocrine hunger signals may also contribute to antibiotic growth gains. Changes in post-meal leptin and ghrelin serum levels, both of which help to regulate satiety, have been associated with the eradication of

Helicobacter pylori following antibiotic treatment (248), although this may play less of a role in food-insecure settings.

An alternative possibility is that antibiotic alteration of the intestinal microbiota may result in growth gains (18,19,147). The intestinal microbiota regulates immune development and inflammation in the gut (128,129), maintains host-microbe homeostasis in the gut (9), and has an important role in nutrient harvesting and absorption (10). Disturbance of intestinal microbiota composition resulting from chronic intestinal colonization with pathogens or overgrowth of commensal bacteria in the small intestine may lead to disruption of these functions. Perturbation of the intestinal microbiota may also lead to intestinal inflammation and increased intestinal permeability. These changes are characteristic of environmental enteropathy, a sub-clinical disorder of the intestinal tract that is ubiquitous in developing countries, and is associated with poor linear growth(78,90,93,210).

Antibiotics are known to induce changes in gut microbiota composition (16,17), and these changes may persist (18,147). Recent work has shown that intestinal microbial taxa may not return to their pre-treatment abundance levels, even after a single exposure to antibiotics (18,147,148), however, the extent of recovery to baseline may depend on the class of antibiotic used (147). A recent review qualitatively summarized the evidence supporting a relationship between antibiotic use and weight, and included evidence from some trials in humans (18). The mechanisms underlying these growth benefits plausibly include resolution of underlying infections or inflammatory processes (for example, environmental enteropathy), and/or alteration of intestinal microbiota composition and function. In an experimental animal model, weight loss in mice resulted from transplantation of donor feces from children with kwashiorkor, but not their healthy twins (14), while increases in total body mass and fat mass were induced in mice transplanted with donor feces from obese adults, but not their lean twins (249). While we cannot rule out an effect of antibiotics on latent bacterial infections in the included trials, it is plausible that the growth benefits we observed also encompass an important intestinal microbiota-mediated growth effect.

Conclusions and Policy Implications

In summary, our results show that antibiotic therapy has a growth-promoting effect, particularly for ponderal growth, in prepubertal children from undernourished populations in low- and middle-income countries. Linear growth appears less responsive to antibiotics. Better understanding the biological mechanisms behind these antibiotic-associated effects on growth is critical for certain populations, such as children under 2 years old (as reversal of stunting beyond this age is challenging (3)), and HIV-infected, HIV-exposed and acutely malnourished children in whom antibiotics continue to be a standard component of care (211,250,251). Antibiotics, however, are not the most viable option for the treatment of malnutrition outside of these highly vulnerable populations in whom antibiotic therapy is already routinely recommended for treatment and prevention of infections. In addition to concerns regarding antimicrobial resistance, antibiotic use has also been associated with adverse events such as antibiotic-associated diarrhea, and the risks of more widespread antibiotic use may not outweigh the benefits. Our findings highlight the co-benefits of antimicrobial therapy that have previously been reported from developing countries (159,161), and provide an intriguing proof-of-concept that treatment of sub-clinical infections and modulation of the intestinal microbiota may have beneficial effects on growth.

11-1 Table 1. Characteristics of the randomized controlled trials of antibiotic use and growth in prepubertal children included in the meta-analysis.

Study [ref]	Publication Year	Indication for Treatment	Eligibility Criteria	Baseline Nutritional Status	Country	Antibiotic Intervention	Control Intervention	Concurrent Intervention
Scrimshaw et al. (244)	1953	Malnutrition	School children	Children in the participating communities averaged 2 to 4 years below the U.S. reference for height & weight	Guatemala	aureomycin	placebo	enriched soya milk powder given 6 days/week except holidays and vacation periods
Guzman et al. (232)	1958	Malnutrition	School children	Children in the participating communities averaged 2 to 4 years below the U.S. reference for height & weight	Guatemala	aureomycin or penicillin	placebo	none
Wolfsdorf et al. (245)	1973	Diarrhea ± Vomiting	Infants presenting with diarrhea or vomiting severe enough to warrant hospitalization	NR	South Africa	trimethoprim-sulphonamide	placebo	"routine" treatment regimens carried out
Gupta et al. (231)	1982	*Giardia lamblia*	Children	Mean percent height- & weight-for-age were 88.6% & 71.5%	Guatemala	metronidazole	placebo	none

Heikens et al. (229)	1993	Malnutrition	Children malnourished according to the Wellcome classification, excluding children with oedema, congenital abnormality, infection requiring hospitalization, or anorexia preventing normal home feeding	Mean percent height- & weight-for-age were 88.6% & 65.1%	Jamaica	metronidazole	none	multivitamins and folic acid, outpatient treatment of infection/illness, advice on breastfeeding and weaning for duration of follow-up
Tahan et al. (158)	2007	Diarrhea	Infants with diarrhea for at least 7 days who needed hospitalization, excluding infants with associated disorders, use of antibiotics in the preceding 7 days, or evidence of systemic infection	Mean height- & weight-for-age Z-scores were -2.02 & -2.36	Brazil	polymixin b	placebo	none
Goto et al. (230)	2009	*Giardia lamblia*	Infants	Mean height- & weight-for-age Z-scores were -1.05 & -1.82	Bangladesh	secnidazole	placebo	none
Trehan et al. (246)	2009	Environmental Enteropathy	Children, excluding children with chronic debilitating illnesses, or evidence of severe acute malnutrition	Mean height- & weight-for-age Z-scores were -1.67 & -0.91	Malawi	rifaximin	placebo	none

Prendergast et al. (161)	2011	OI Prophylaxis	Children with a positive HIV antibody test, excluding children with an OI, life expectancy ≤4 weeks, current co-trimoxazole treatment or allergy to this drug, or previous *P.jirovecii* pneumonia	Mean height- & weight-for-age Z-scores were -3.55 & -3.10	Zambia	co-trimoxazole	placebo	none
Trehan et al. (159)	2013	SAM	Children with edema, weight-for-height z-score < -3, or both	Mean height-for-age z-score was -3.19	Malawi	amoxicillin or cefdinir	placebo	standardized nutrition counseling and RUTF at a dose of approximately 175 kcal/kg/day given in 2 week intervals

Abbreviations: OI, opportunistic infection; NR, not reported; HIV, Human Immunodeficiency Virus, SAM, severe acute malnutrition; RUTF, ready-to-use therapeutic food; kcal, kilocalories.

11-2. Table 2. Growth outcomes and potential treatment effect moderators in the randomized controlled trials of antibiotic use and growth in prepubertal children included in the meta-analysis.

									Mean growth/month of follow-up			
									Height(cm)		Weight(g)	
Study [ref]	IPD	Mean Age ± sd (months)	No. Male (%)	Antibiotic Class, Spectrum	Dosage	Doses/ day[d]	Days treated	Mean Follow-up (days)	Co	Tx	Co	Tx
Scrimshaw et al. (244)	No	114.9±NR[a]	143 (57.2)[a]	bacteriostatic, broadspectrum	50mg	1	667[c]	758	0.39	0.42	180.0	270.0
Guzman et al. (232)	No	114.9±NR	143 (57.2)	bacteriostatic, broadspectrum	50mg	1	394[c]	394	0.36	0.36	170.0	166.0
Wolfsdorf et al. (245)	No	5.9±6.4	NR	bactericidal, broadspectrum	NR	NR	NR	91	NR	NR	664.0	788.4
Gupta et al. (231)	No	23.0±17.2[b]	NR	bactericidal, narrow-spectrum	25mg/kg	2	42	NR	0.51	0.58	135.9	154.2
Heikens et al. (229)	No	14.1±6.5	NR	bactericidal, narrow-spectrum	20mg/kg	1	5	179	12.40	12.20	1,336.7	1,393.3
Tahan et al. (158)	Yes	4.0±2.0	17 (68.0)	bactericidal, narrow-spectrum	2.5mg/kg	4	7	7	NR	NR	710.5[e]	735.7[e]
Goto et al. (230)	Yes	8.6±3.2	135 (50.4)	bactericidal, narrow-spectrum	35mg/kg	1	10	264	9.11	9.12	1,105.1	1,100.7
Trehan et al. (246)	Yes	47.2±7.12	60 (41.7)	bacteriostatic, broadspectrum	10 mg	2	7	28	107.56[f]	107.96[f]	14,957.1[f]	15,030.3[f]

Prendergast et al. (161)	Yes	64.5±44.7	266 (49.2)	bactericidal, broadspectrum	240g (<5yrs); 480g (>5yrs)	1	575	575	5.66	5.77	803.9	845.0
Trehan et al. (159)	Yes	21.1±9.1	1,317 (47.6)	bactericidal, broadspectrum	7mg/kg (cefdinir); 40-45mg/kg (amoxicillin)	2	7	43	26.74	26.76	2,898.0	2,938.9

Abbreviations: IPD, individual patient data; NR, not reported; No., number; sd, standard deviation; mg, milligram; kg, kilogram; cm, centimeters; g, grams; Co, controls; Tx, treated.

[a]Not reported by Scrimshaw *et al.* (244) We assumed these values were the same as in Guzman *et al.* (232) since both studies were conducted in communities in the Guatemlan highlands in the 1950s by the same research group and recruited children in the 5-12 year age range.

[b]Not reported by Gupta *et al.* (231), estimated from Scrimshaw *et al.* 1968 (252)

[c]Estimated from the mean number of treatment days reported per trial arm.

[d]Number of doses per day.

[e]Mean change in weight per day; follow-up was seven days.

[f]Follow-up was 28 days; these represent height and weight at the end of follow-up.

11-3. Table 3. Estimated average differences in antibiotic treatment effects on growth in prepubertal children, using weighted bivariate random-effects meta-regression.

		Height (cm/month)				Weight (g/month)		
Trial Characteristics	**n**	**Mean Difference**	**p-value**	***I^2(95%CI)***	**n**	**Mean Difference**	**p-value**	***I^2(95%CI)***
Geographic Region (Africa vs other)	8	0.05	0.275	79.6%(39.7 to 98.8)	10	35.57	0.002	50.9%(10.6 to 99.1)
Publication Year	8	0.00	0.650	78.7%(35.5 to 99.0)	10	0.50	0.275	77.6%(47.1 to 99.9)
Treatment effect adjusted for baseline imbalances (yes vs no)	8	0.00	0.964	88.5%(55.9 to 99.9)	10	-17.59	0.465	85.4%(62.8 to 99.9)
Mean Length of Follow-up (days)	7	0.00	0.282	79.8%(35.3 to 99.4)	9	-0.05	0.490	87.5%(61.1 to 99.9)
Number of Doses/day	8	0.02	0.648	83.4%(44.1 to 98.8)	9	21.70	0.307	84.4%(56.9 to 99.7)
Duration of treatment (days)	8	0.00	0.340	81.9%(47.1 to 99.1)	9	0.00	0.921	86.6%(65.6 to 99.9)
Antibiotic Class (bactericidal vs bacteriostatic)[a]	7	-0.05	0.792	71.6%(25.9 to 99.6)	8	-51.61	0.727	87.1%(72.1 to 100.0)
Antibiotic Spectrum (broad-spectrum vs narrow)	8	0.02	0.666	89.2%(57.2 to 99.3)	10	9.41	0.666	84.6%(61.0 to 99.9)
Subjects given a concurrent nutritional intervention (yes vs no)	8	-0.05	0.356	82.5%(44.9 to 98.6)	10	31.00	0.110	75.7%(44.2 to 99.9)
Mean Age (months)	8	0.00	0.948	82.0%(1.7 to 96.7)	10	-0.24	0.381	82.0%(54.3 to 99.9)
Treatment was for malnutrition (yes vs no)	8	-0.06	0.066	75.2%(17.4 to 99.0)	10	2.65	0.906	85.1%(62.5 to 99.9)
Treatment was for *Giardia lamblia* infection (yes vs no)	8	0.01	0.833	88.6%(56.2 to 99.4)	10	-26.42	0.210	82.2%(55.1 to 99.9)
Treatment was for diarrhea±vomiting (yes vs no)[b]	NA	NA	NA	NA	10	144.37	0.075	85.3%(60.9 to 99.8)

Abbreviations: n, number of trials included in the meta-regression model; NA, not applicable; cm, centimeters; g, grams; I^2, I-squared statistic; 95%CI, 95% confidence interval.

[a]Excludes Prendergast *et al*. and Wolfsdorf *et al*. because it is unclear whether trimethoprim with sulphonamide or sulfamethoxazole are bacteriostatic or bactericidal in combination.

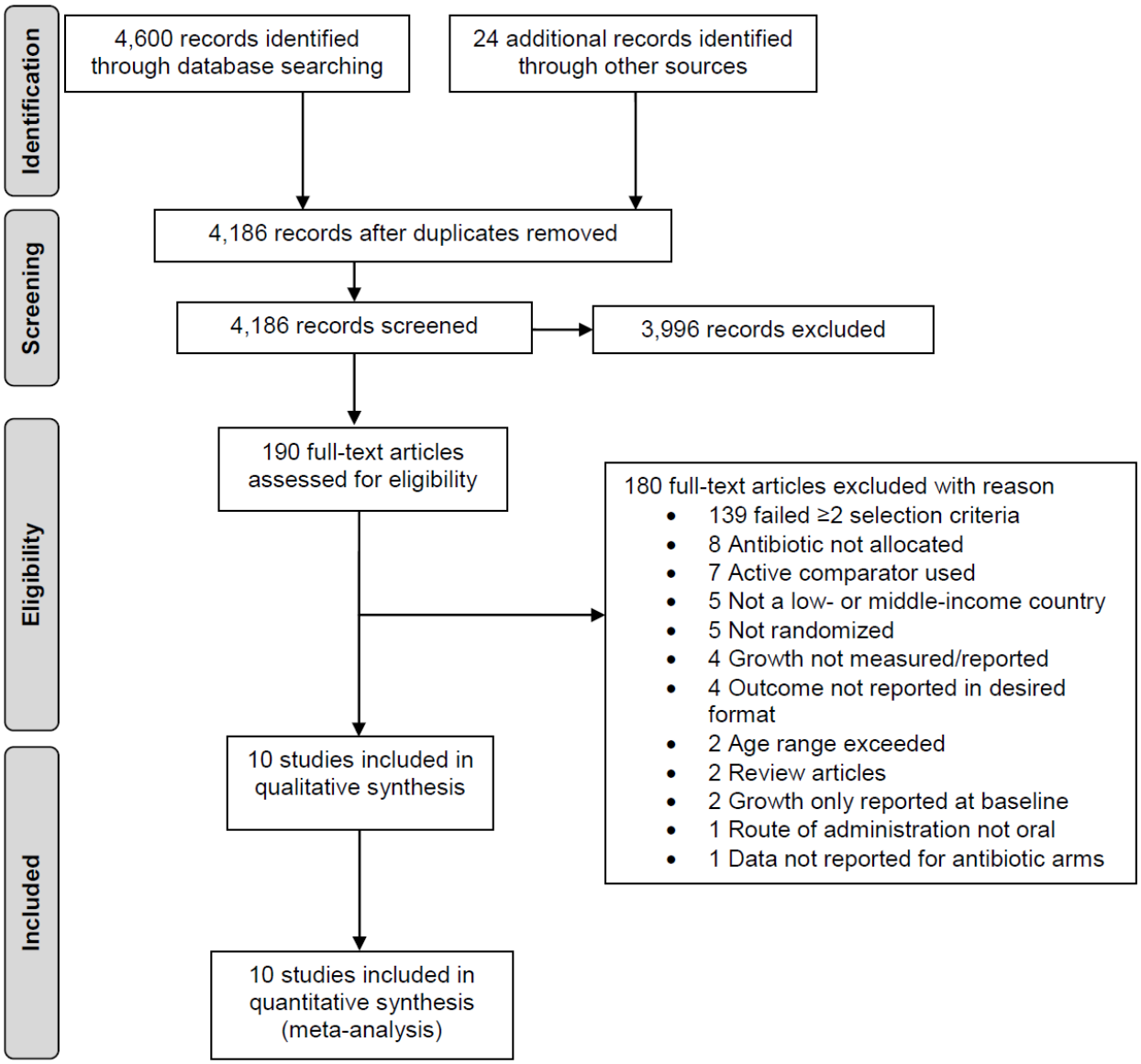
[b]No trials reporting height treated participants for diarrhea±vomitting.

11-4. Table 4. Significant moderators of antibiotic treatment effects on growth in prepubertal children, using weighted random-effects meta-regression adjusted for mean study population age.

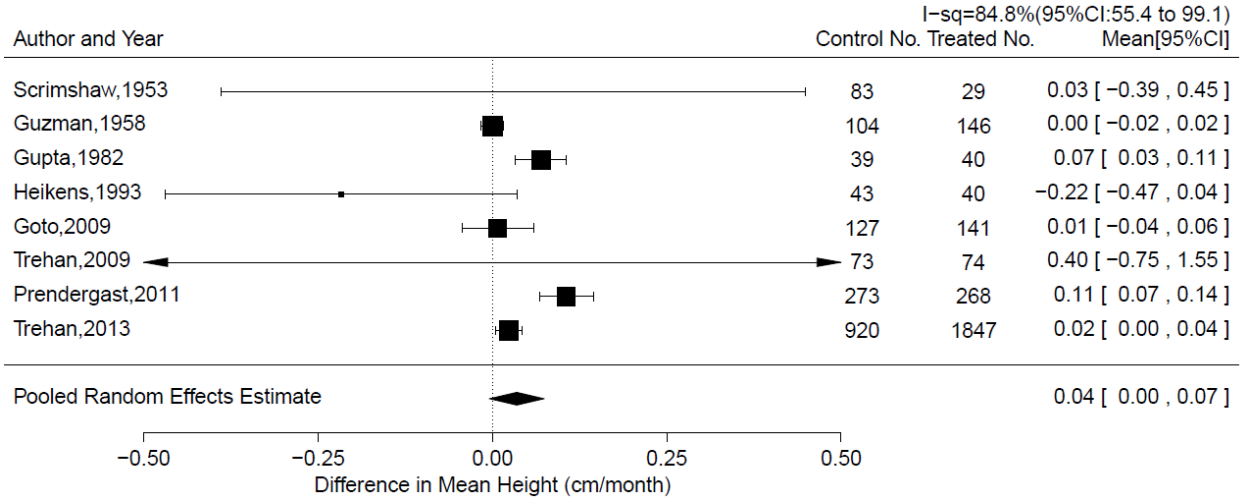
Trial Characteristics	n	Mean Difference (95%CI)	I^2(95%CI)
Height Model 1 (cm/month)			
Duration of Treatment (days)	8	0.007 (0.00 to 0.01)	53.6%(0.0 to 99.3)
Mean age (months)		-0.001(-0.002 to 0.00)	
Weight Model 1 (g/month)			
Geographic Region (Africa vs other)	10	33.2 (5.3 to 61.2)	53.5%(3.6 to 99.9)
Mean age (months)		-0.2 (-0.4 to -0.1)	
Weight Model 2 (g/month)			
Treatment was for *Giardia lamblia* (yes vs no)	10	-46.9 (-83.2 to -10.6)	57.8%(9.3 to 99.9)
Mean age (months)		-0.5 (-1.0 to -0.1)	

Abbreviations: n, number of trials included in the meta-regression model; cm, centimeters; g, grams; I^2, I-squared statistic; 95%CI, 95% confidence interval.

11-1 Figure 1. Flow diagram of search retrieval and trial selection.

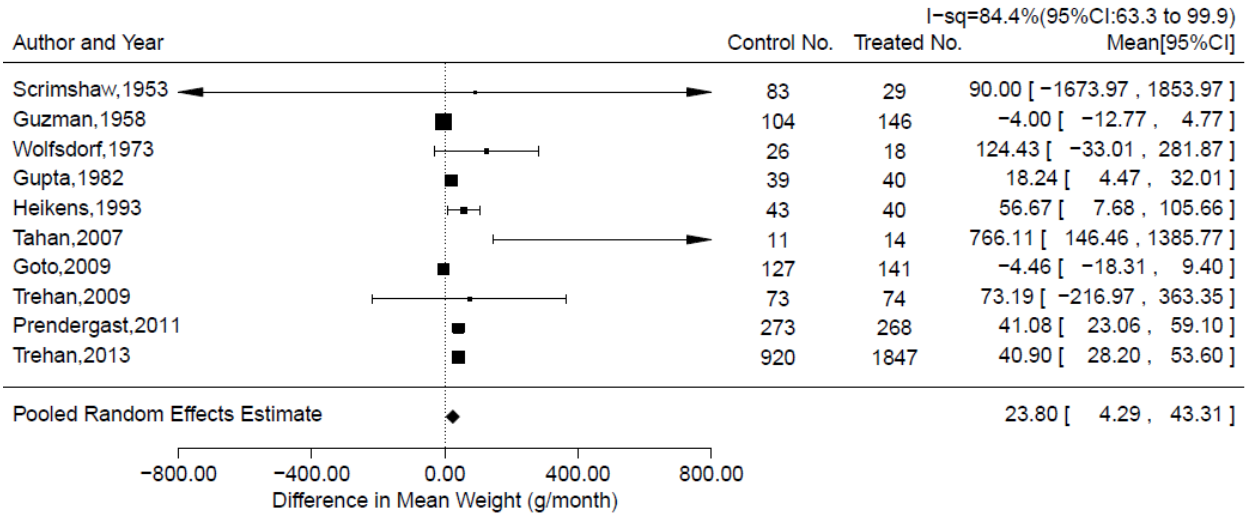


11-2. Figure 2. Random effects meta-analyses and forest plots of antibiotic use and height.



No, number; I^2, I squared statistic; 95%CI, 95% confidence interval; cm, centimeters. Point size reflects study weight.

11-3. Figure 3. Random effects meta-analyses and forest plots of antibiotic use and weight.



No., number; I^2, I squared statistic; 95%CI, 95% confidence interval; g, grams. Point size reflects study weight.

11.3. Appendix 1: Search Strings

Medline

1. exp Anti-Bacterial Agents/
2. (antibiotic or antimicrobial or anti-infective or antibacterial).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
3. (amikacin or gentamicin or kanamycin or neomycin or netilmicin or tobramycin or paromomycin or aminoglycosides).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
4. (Geldanamycin or Herbimycin or Ansamycins or Carbacephem or Loracarbef or Carbapenems or Ertapenem or Doripenem or Imipenem or Cilastatin or Meropenem).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
5. (Cephalosporins or Cefadroxil or Cefazolin or Cefalotin or Cefalothin or Cefalexin or Cefaclor or Cefamandole or Cefoxitin or Cefprozil or Cefuroxime or Cefixime or Cefdinir or Cefditoren or Cefoperazone or Cefotaxime or Cefpodoxime or Ceftazidime or Ceftibuten or Ceftizoxime or Ceftriaxone or Cefepime or Ceftobiprole).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
6. (Glycopeptides or Teicoplanin or Vancomycin or Telavancin or Lincosamides or Clindamycin or Lincomycin or Lipopeptide or Daptomycin or Macrolides or Azithromycin or Clarithromycin or Dirithromycin or Erythromycin or Roxithromycin or Troleandomycin or Telithromycin or Spectinomycin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
7. (Monobactams or Aztreonam or Nitrofurans or Furazolidone or Nitrofurantoin or Penicillins or Amoxicillin or Ampicillin or Azlocillin or Carbenicillin or Cloxacillin or Dicloxacillin or Flucloxacillin or Mezlocillin orMethicillin or Nafcillin or Oxacillin or "Penicillin G" or "Penicillin V" or Piperacillin or Temocillin or Ticarcillin or Amoxicillin or clavulanate or Ampicillin or sulbactam or Piperacillin or tazobactam or Ticarcillin or clavulanate).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
8. (Polypeptides or Bacitracin or Colistin or "Polymyxin B" or Quinolones or Ciprofloxacin or Enoxacin or Gatifloxacin or Levofloxacin or Lomefloxacin or Moxifloxacin or Nalidixic acid or Norfloxacin or Ofloxacin or Trovafloxacin or Grepafloxacin or Sparfloxacin or Temafloxacin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
9. (Sulfonamides or Mafenide or Sulfonamidochrysoidine or Sulfacetamide or Sulfadiazine or Silver sulfadiazine or Sulfamethizole or Sulfamethoxazole or Sulfanilimide or Sulfasalazine or Sulfisoxazole or Trimethoprim or Co-trimoxazole or cotrimoxazole).mp. [mp=title, abstract,

original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]

10. (tetracyclines or Demeclocycline or Doxycycline or Minocycline or Oxytetracycline or Tetracycline or Arsphenamine or Chloramphenicol or Fosfomycin or Fusidic acid or Linezolid or Metronidazole or Mupirocin or Platensimycin or Quinupristin or Dalfopristin or Rifaximin or Thiamphenicol or Tigecycline or Tinidazole).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
11. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10
12. child nutrition disorders/ or infant nutrition disorders/
13. Protein-Energy Malnutrition/
14. Nutritional Status/
15. Deficiency Diseases/
16. Anthropometry/
17. (undern* or maln* or underweight or wasted or wasting or stunted or stunting or "growth faltering" or "weight gain" or "growth velocity" or "height gain" or "growth faltering" or "growth retardation" or "growth deficit").mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
18. exp Body Size/
19. "body weights and measures"/ or body mass index/ or body size/
20. Malnutrition/
21. (z score or z-score).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
22. 12 or 13 or 14 or 15 or 17 or 20
23. 16 or 18 or 19 or 21
24. 22 or 23
25. 11 and 24
26. randomized controlled trial.pt. or randomized.mp. or placebo.mp.
27. 25 and 26
28. limit 27 to humans
29. remove duplicates from 28

Embase

1. exp antiinfective agent/
2. (amikacin or gentamicin or kanamycin or neomycin or netilmicin or tobramycin or paromomycin or aminoglycosides).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
3. (Geldanamycin or Herbimycin or Ansamycins or Carbacephem or Loracarbef or Carbapenems or Ertapenem or Doripenem or Imipenem or Cilastatin or Meropenem).mp.

[mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
4. (Cephalosporins or Cefadroxil or Cefazolin or Cefalotin or Cefalothin or Cefalexin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
5. (Cefaclor or Cefamandole or Cefoxitin or Cefprozil or Cefuroxime or Cefixime or Cefdinir or Cefditoren or Cefoperazone or Cefotaxime or Cefpodoxime or Ceftazidime or Ceftibuten or Ceftizoxime or Ceftriaxone or Cefepime or Ceftobiprole).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
6. (Glycopeptides or Teicoplanin or Vancomycin or Telavancin or Lincosamides or Clindamycin or Lincomycin or Lipopeptide or Daptomycin or Macrolides or Azithromycin or Clarithromycin or Dirithromycin or Erythromycin or Roxithromycin or Troleandomycin or Telithromycin or Spectinomycin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
7. (Monobactams or Aztreonam or Nitrofurans or Furazolidone or Nitrofurantoin or Penicillins or Amoxicillin or Ampicillin or Azlocillin or Carbenicillin or Cloxacillin or Dicloxacillin or Flucloxacillin or Mezlocillin or Methicillin or Nafcillin or Oxacillin or "Penicillin G" or "Penicillin V" or Piperacillin or Temocillin or Ticarcillin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
8. (Amoxicillin or clavulanate or Ampicillin or sulbactam or Piperacillin or tazobactam or Ticarcillin or clavulanate or Polypeptides or Bacitracin or Colistin or "Polymyxin B").mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
9. (Quinolones or Ciprofloxacin or Enoxacin or Gatifloxacin or Levofloxacin or Lomefloxacin or Moxifloxacin or Nalidixic acid or Norfloxacin or Ofloxacin or Trovafloxacin or Grepafloxacin or Sparfloxacin or Temafloxacin).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
10. (Sulfonamides or Mafenide or Sulfonamidochrysoidine or Sulfacetamide or Sulfadiazine or Silver sulfadiazine or Sulfamethizole or Sulfamethoxazole or Sulfanilimide or Sulfasalazine or Sulfisoxazole or Trimethoprim or Co-trimoxazole or cotrimoxazole).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
11. (tetracyclines or Demeclocycline or Doxycycline or Minocycline or Oxytetracycline or Tetracycline).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
12. (Arsphenamine or Chloramphenicol or Fosfomycin or Fusidic acid or Linezolid or Metronidazole or Mupirocin or Platensimycin or Quinupristin or Dalfopristin or Rifaximin or Thiamphenicol or Tigecycline or Tinidazole).mp. [mp=title, abstract, original title, name of

substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]

13. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12
14. exp nutritional disorder/
15. nutritional disorder/ or exp malnutrition/
16. nutritional disorder/ or exp nutritional deficiency/
17. exp protein calorie malnutrition/
18. exp anthropometry/
19. exp arm circumference/ or exp body height/ or exp body mass/ or exp body size/ or exp body weight/ or exp growth curve/ or exp head circumference/
20. (undern* or maln* or underweight or wasted or wasting or stunted or stunting or "growth faltering" or "weight gain" or "growth velocity" or "height gain" or "growth faltering" or "growth retardation" or "growth deficit").mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
21. (z score or z-score).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier]
22. exp underweight/ or exp body weight disorder/
23. exp growth retardation/
24. exp child growth/ or exp postnatal growth/
25. 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26. 13 and 25
27. random:.tw. or placebo:.mp. or double-blind:.tw.
28. 26 and 27
29. limit 28 to human

Scopus

TITLE-ABS-KEY(anti-bacterial agents OR antibiotic* OR antimicrobial* OR anti-infectiv* OR antibacterial OR anti-bacterial OR amikacin OR gentamicin OR kanamycin OR neomycin OR netilmicin OR tobramycin OR paromomycin OR aminoglycosides OR geldanamycin OR herbimycin OR ansamycins OR carbacephem OR loracarbef OR carbapenems OR ertapenem OR doripenem OR imipenem OR cilastatin OR meropenem OR geldanamycin OR herbimycin OR ansamycins OR carbacephem OR loracarbef OR carbapenems OR ertapenem OR doripenem OR imipenem OR cilastatin OR meropenem OR cephalosporins OR cefadroxil OR cefazolin OR cefalotin OR cefalothin OR cefalexin OR cefaclor OR cefamandole OR cefoxitin OR cefprozil OR cefuroxime OR cefixime OR cefdinir OR cefditoren OR cefoperazone OR cefotaxime OR cefpodoxime OR ceftazidime OR ceftibuten OR ceftizoxime OR ceftriaxone OR cefepime OR ceftobiprole OR glycopeptides OR teicoplanin OR vancomycin OR telavancin OR lincosamides OR clindamycin OR lincomycin OR lipopeptide OR daptomycin OR macrolides OR azithromycin OR clarithromycin OR dirithromycin OR erythromycin OR roxithromycin OR troleandomycin OR telithromycin OR spectinomycin OR monobactams OR aztreonam OR nitrofurans OR furazolidone OR nitrofurantoin OR penicillins OR amoxicillin OR ampicillin OR azlocillin OR

carbenicillin OR cloxacillin OR dicloxacillin OR flucloxacillin OR mezlocillin OR methicillin OR nafcillin OR oxacillin OR "Penicillin G" OR "Penicillin V" OR piperacillin OR temocillin OR ticarcillin OR amoxicillin OR clavulanate OR ampicillin OR sulbactam OR piperacillin OR tazobactam OR ticarcillin OR clavulanate OR polypeptides OR bacitracin OR colistin OR "Polymyxin B" OR quinolones OR ciprofloxacin OR enoxacin OR gatifloxacin OR levofloxacin OR lomefloxacin OR moxifloxacin OR "Nalidixic acid" OR norfloxacin OR ofloxacin OR trovafloxacin OR grepafloxacin OR sparfloxacin OR temafloxacin OR sulfonamides OR mafenide OR sulfonamidochrysoidine OR sulfacetamide OR sulfadiazine OR sulfamethizole OR sulfamethoxazole OR sulfanilimide OR sulfasalazine OR sulfisoxazole OR trimethoprim OR co-trimoxazole OR cotrimoxazole OR tetracyclines OR demeclocycline OR doxycycline OR minocycline OR oxytetracycline OR tetracycline OR arsphenamine OR chloramphenicol OR fosfomycin OR "Fusidic acid" OR linezolid OR metronidazole OR mupirocin OR platensimycin OR quinupristin OR dalfopristin OR rifaximin OR thiamphenicol OR tigecycline OR tinidazole) AND TITLE-ABS-KEY("child nutrition disorders" OR "infant nutrition disorders" OR malnutrition OR "Protein-Energy Malnutrition" OR "Nutritional Status" OR "Deficiency Diseases" OR anthropometry OR undernutrition OR undernourished OR malnourished OR underweight OR wasted OR wasting OR stunted OR stunting OR "growth faltering" OR "weight gain" OR "growth velocity" OR "height gain" OR "growth faltering" OR "growth retardation" OR "growth deficit" OR "body weights and measures" OR "body mass index" OR "body size" OR "z score" OR z-score)

Cochrane Central Register of Controlled Trials (Clinical Trials)

1. (Anti-Bacterial Agents OR antibiotic* or antimicrobial* or anti-infectiv* or antibacterial or anti-bacterial OR amikacin or gentamicin or kanamycin or neomycin or netilmicin or tobramycin or paromomycin or aminoglycosides OR Geldanamycin or Herbimycin or Ansamycins or Carbacephem or Loracarbef or Carbapenems or Ertapenem or Doripenem or Imipenem or Cilastatin or Meropenem OR Geldanamycin or Herbimycin or Ansamycins or Carbacephem or Loracarbef or Carbapenems or Ertapenem or Doripenem or Imipenem or Cilastatin or Meropenem OR Cephalosporins or Cefadroxil or Cefazolin or Cefalotin or Cefalothin or Cefalexin or Cefaclor or Cefamandole or Cefoxitin or Cefprozil or Cefuroxime or Cefixime or Cefdinir or Cefditoren or Cefoperazone or Cefotaxime or Cefpodoxime or Ceftazidime OR Ceftibuten or Ceftizoxime or Ceftriaxone or Cefepime or Ceftobiprole OR Glycopeptides or Teicoplanin or Vancomycin or Telavancin or Lincosamides or Clindamycin or Lincomycin OR Lipopeptide or Daptomycin or Macrolides or Azithromycin or Clarithromycin or Dirithromycin or Erythromycin or Roxithromycin or Troleandomycin or Telithromycin or Spectinomycin OR Monobactams or Aztreonam or Nitrofurans or Furazolidone or Nitrofurantoin or Penicillins or Amoxicillin or Ampicillin or Azlocillin or Carbenicillin or Cloxacillin or Dicloxacillin OR Flucloxacillin or Mezlocillin or Methicillin or Nafcillin or Oxacillin or "Penicillin G" or "Penicillin V" or Piperacillin or Temocillin or Ticarcillin OR Amoxicillin or clavulanate or Ampicillin or sulbactam or Piperacillin or tazobactam or Ticarcillin or clavulanate OR Polypeptides or Bacitracin or Colistin or "Polymyxin B" or Quinolones or Ciprofloxacin or Enoxacin or Gatifloxacin or Levofloxacin or Lomefloxacin or Moxifloxacin or "Nalidixic acid" OR Norfloxacin or Ofloxacin or

Trovafloxacin or Grepafloxacin or Sparfloxacin or Temafloxacin OR Sulfonamides or Mafenide or Sulfonamidochrysoidine or Sulfacetamide or Sulfadiazine or Sulfamethizole or Sulfamethoxazole or Sulfanilimide or Sulfasalazine or Sulfisoxazole or Trimethoprim or Co-trimoxazole or cotrimoxazole OR tetracyclines or Demeclocycline or Doxycycline or Minocycline or Oxytetracycline or Tetracycline or Arsphenamine or Chloramphenicol or Fosfomycin or "Fusidic acid" or Linezolid OR Metronidazole or Mupirocin or Platensimycin or Quinupristin or Dalfopristin or Rifaximin or Thiamphenicol or Tigecycline or Tinidazole):ti,ab,kw in Clinical Trials

2. "child nutrition disorders" or "infant nutrition disorders" or malnutrition OR "Protein-Energy Malnutrition" OR "Nutritional Status" OR "Deficiency Diseases" OR Anthropometry OR undernutrition OR undernourished or malnourished or underweight or wasted or wasting or stunted or stunting or "growth faltering" or "weight gain" or "growth velocity" or "height gain" or "growth faltering" or "growth retardation" or "growth deficit" OR "body weights and measures" or "body mass index" or "body size" OR "z score" or z-score:ti,ab,kw
3. (#1 AND #2)

11.4. Appendix 2: Gough EK, Moodie EE, Prendergast AJ, et al. The impact of antibiotics on growth in children in low and middle income countries: systematic review and meta-analysis of randomised controlled trials. BMJ 2014;348:g2267.

BMJ

BMJ 2014;348:g2267 doi: 10.1136/bmj.g2267 (Published 15 April 2014)

RESEARCH

The impact of antibiotics on growth in children in low and middle income countries: systematic review and meta-analysis of randomised controlled trials

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Abstract

Objectives To determine whether antibiotic treatment leads to improvements in growth in prepubertal children in low and middle income countries, to determine the magnitude of improvements in growth, and to identify moderators of this treatment effect.

Design Systematic review and meta-analysis.

Data sources Medline, Embase, Scopus, the Cochrane central register of controlled trials, and Web of Science.

Study selection Randomised controlled trials conducted in low or middle income countries in which an orally administered antibacterial agent was allocated by randomisation or minimisation and growth was measured as an outcome. Participants aged 1 month to 12 years were included. Control was placebo or non-antimicrobial intervention.

Results Data were pooled from 10 randomised controlled trials representing 4316 children, across a variety of antibiotics, indications for treatment, treatment regimens, and countries. In random effects models, antibiotic use increased height by 0.04 cm/month (95% confidence interval 0.00 to 0.07) and weight by 23.8 g/month (95% confidence interval 4.3 to 43.3). After adjusting for age, effects on height were larger in younger populations and effects on weight were larger in African studies compared with other regions.

Conclusion Antibiotics have a growth promoting effect in prepubertal children in low and middle income countries. This effect was more pronounced for ponderal than for linear growth. The antibiotic growth promoting effect may be mediated by treatment of clinical or subclinical infections or possibly by modulation of the intestinal microbiota. Better definition of the mechanisms underlying this effect will be important to inform optimal and safe approaches to achieving healthy growth in vulnerable populations.

Introduction

Undernutrition in early childhood, characterised by poor linear or ponderal growth, underlies approximately one third of all mortality in children aged under 5 years worldwide.[1] Linear growth, measured as height or length, is an indicator of long term nutritional status; children whose height for age is more than 2 standard deviations below the reference population mean

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Extra material supplied by the author (see http://www.bmj.com/content/348/bmj.g2267?tab=related#webextra)

Search strings
Review protocol

are termed stunted. Ponderal growth, measured as body weight, is viewed as an indicator of short term or long term nutritional status. Children whose weight for age is more than 2 standard deviations below the reference population mean are termed underweight. Underweight and stunting, particularly during the first two years of life, have short term effects on morbidity and mortality and long term effects on cognition, educational achievement, and economic productivity as an adult.[2] Given the current global focus on reducing underweight and stunting to reach forthcoming global health targets,[3 4] interest in evaluating interventions to promote healthy growth in childhood is increasing.[5] Primary interventions to improve growth in children have largely focused on nutritional supplementation and prevention of diarrhoea. However, the impact of these interventions on restoring growth deficits in undernourished children is modest.[6-9] Restoration of deficits in linear growth is particularly challenging beyond the first two years of life.[2]

The growth promoting effects of antibiotics were first observed in animals in the 1940s. Small daily doses of broad spectrum antibiotics have been found to improve average daily weight gain in farm animals by as much as 73%.[10-18] These observations led to the hypothesis that food animals reared in conditions of poor sanitation and hygiene have impaired growth because of chronic exposure to environmental microbes and pathogens, and that antibiotic treatment may therefore improve growth.[19]

In humans, an association between infections and malnutrition in children is supported in the literature.[20 21] Nutrient harvesting from the diet and the inflammatory response of the gut are also modulated by the intestinal microbiota, a microbial ecosystem that is essential to human health and nutrition.[22-26] Perturbation of this microbial community through chronic exposure to environmental microbes or pathogens may also be detrimental to growth in children,[19 27-29] and studies have shown that antibiotic use can affect the composition of the microbial community.[30 31] Antibiotic use has also been associated with significant height and weight gains among children in some target populations.[32-35] However, results have not always been consistent,[32 36-39] and researchers continue to investigate the potential co-benefits of antibiotic treatment on growth in children.[40 41]

We carried out a systematic review of randomised controlled trials to determine whether improvements in growth are seen among prepubertal children (1 month to 12 years old) treated with antibiotics in low and middle income countries; to determine the magnitude of these growth effects; and to identify moderators of this treatment effect. We hypothesised that antibiotics would have a positive average effect on both height and weight, and that treatment effect size would be moderated by the characteristics of antibiotic treatment, differences in study population, and trial quality.

Methods

Search strategy and selection criteria

This review is reported in accordance with the PRISMA statement[42] and recommendations for reporting meta-analyses of individual patient data.[43] We searched Medline (including In-Process and Other Non-Indexed Citations) and Embase, both using Ovid, as well as Scopus and the Cochrane central register of controlled trials up to 12 December 2013. A professional librarian helped to develop the search strings (see supplementary appendix 1 for details of search strings and appendix 2 for the review protocol).

We searched for randomised controlled trials conducted in low or middle income countries with participants aged 1 month to 12 years allocated by randomisation or minimisation to antibacterial treatment given by mouth, or to control. Control interventions included placebo, an intervention with no known antimicrobial effect, or no treatment. We selected trials, published or unpublished, if growth was measured as an outcome. Studies of anthelmintic treatments were excluded, since systematic reviews of such trials have already been conducted.[44 45] We placed no restrictions on language, year of publication, or the length of follow-up, and excluded quasiexperimental studies, observational studies, reviews, and simulations. We excluded studies of neonates (<1 month old) since growth patterns during the neonatal period, particularly among preterm infants, are different from the post-neonatal period. Finally, we considered trials ineligible for inclusion if the condition being treated did not depend on the antimicrobial effect of antibiotic treatment (for example, use of specific antibiotics to reduce feeding intolerance through prokinetic effects or to improve lung function through anti-inflammatory effects).

Two investigators (EKG and SMAJ) independently assessed titles and abstracts for eligible publications. If eligibility could not be determined, the full article was retrieved and the methods screened. In an effort to find similar trials we used Web of Science to search for publications that cited the included studies, and we also handsearched reference lists of included trials and any review articles identified. A third investigator (AJP) adjudicated discrepancies.

Data abstraction and analysis

Study quality was determined by assessing the included publications for risk of bias from the procedures used for sequence generation, allocation concealment, and blinding; and by informative censoring or selective outcome reporting using a standardised instrument adapted from the Cochrane handbook.[46] Two reviewers (EKG and SMAJ) independently assessed the included publications. Discrepancies were resolved by consensus.

We contacted study authors up to three times by email (or by telephone if email was unsuccessful) to determine their interest in collaborating on this review and to request individual patient data. When such data could not be obtained, the same two reviewers independently abstracted data using a standardised pretested form, with discrepancies resolved by consensus. For each trial arm we abstracted number of participants, number lost to follow-up or excluded after randomisation, mean baseline height or weight, and mean height or weight (and standard deviations) at the end of follow-up. For reported treatment effects we also abstracted P values, confidence intervals, and standard errors. Where mean change in height or weight for each unit of follow-up time was reported, we retrieved the same information. We also abstracted several trial level characteristics, which we defined a priori as potential moderators of treatment effect: indication for treatment, country, proportion boys, mean age, antibiotic agent, dosage, frequency and duration of antibiotic treatment, concurrent interventions, length of follow-up, and whether treatment effects were adjusted for imbalances at baseline. We defined antibiotic class as bacteriostatic or bactericidal, and antibiotic spectrum as broad or narrow. Broad spectrum antibiotics were defined as those reported in the literature to be effective against a wide range of Gram positive and Gram negative bacteria, and narrow spectrum antibiotics as those reported in the literature to be effective

against a limited range of bacteria.[47-55] Risk of bias domains were treated as potential sources of heterogeneity.

Outcomes were mean height (cm) or weight (g) at the end of follow-up or mean change in height (cm) or weight (g) per unit of follow-up time. The difference in means between treatment and control arms was the measure of treatment effect. We scaled the treatment effects and their variances as the average effect for each month of follow-up. We analysed height and weight separately. When a trial allocated participants to more than one intervention, we abstracted data from the arm allocated to receive antibiotics.[56-58] When a trial allocated participants to more than one antibiotic arm, we combined the data from both arms to avoid unit of analysis errors.[34 59]

We combined individual patient data and aggregate data using a two step approach.[60] In the first step we estimated treatment effects in each trial with individual patient data in an intention to treat analysis using linear mixed models to allow for random intercepts and serial correlation. For each trial we fit one individual patient data model, with baseline growth, age, sex, duration of follow-up, and a duration by treatment interaction included as covariates. In the second step we used a random effects model to pool intention to treat effect estimates (obtained from separate individual patient data trials in step 1) with intention to treat effect estimates abstracted from publications with aggregate data.

We assessed statistical heterogeneity using the I^2 statistic.[61] Heterogeneity was explored using weighted metaregression and subgroup meta-analyses. Statistical significance was evaluated at $\alpha<0.05$. We assessed publication bias using Egger's test.[61] Sensitivity analyses were performed in two ways: to determine the robustness of meta-analysis results to the removal of studies,[62] and by fitting linear mixed models restricted to the five trials for which individual patient data were available.

Trials with individual patient data were modelled using the lme4 package, and we fit all meta-analyses and metaregression models using the metafor package,[63] both using R version 2.15.1.

Results

Study selection

The electronic search identified 4600 records. An additional 24 records were identified through Web of Science and a backward search of reference lists (fig 1⇓). Of these, 190 studies were retrieved and screened for eligibility. Overall, 139 studies failed to meet at least two selection criteria. Thirty four additional studies were excluded for failing to meet one of the following criteria: no antibiotic was allocated (n=8), an active comparator was used (n=7), the trial was not conducted in a low or middle income country (n=5), treatment was not randomised (n=5), growth was not measured or reported (n=4), participants' age range exceeded 12 years (n=2), review articles (n=2), and the antibiotic was not administered orally (n=1). Only four were non-English language texts. These were screened using an electronic translator.

Four additional trials were excluded because they reported differences in prevalence of stunting or wasting,[64-66] or reported growth using the Wetzel grid method.[67] Of these, three authors could not be reached to request individual patient data or unpublished data,[64 66 67] and data were no longer available from one.[65] Another author was contacted and provided individual patient data but did not provide a data dictionary. Since this publication did not report outcomes for the antibiotic arms, this trial was excluded.[68] Of these five otherwise eligible randomised controlled trials that were excluded because growth was not reported in the desired format, four reported no growth benefits from antibiotics,[64-66 68] but they would only have represented 8% of the total person time if they were included and would not have greatly influenced our findings. Another two trials were excluded because they only reported growth at baseline and the authors could not be reached.[69 70]

Published data were available from five trials,[56 58 59 71 72] and complete datasets for individual patient data were obtained from five trials.[33-35 57 73] Thus 10 randomised controlled trials were included in the meta-analysis.[33-35 56-59 71-73] Only data from the secnidazole and placebo arms were included for Goto and colleagues,[57] from the metronidazole and placebo arms for Gupta and colleagues,[58] and from the metronidazole and no intervention arms for Heikens and colleagues.[56]

Study characteristics

Of these 10 trials, nine were placebo controlled and one gave the controls no treatment.[56] Indication for treatment varied by trial and included malnutrition (n=4), infection with *Giardia lamblia* (n=2), diarrhoea with or without vomiting (n=2), environmental enteropathy (n=1), and prophylaxis in children infected with human immunodeficiency virus (n=1). The earliest included trial was published in 1953 and the most recent in 2013. Three trials gave a nutritional supplement to participants in both arms[34 56 71] in addition to antibiotics or control (table 1⇓). Only three trials with aggregate data reported the number of male participants (table 2⇓). Two trials recruited children admitted to hospital, and both reported weight only.[33 72]

Eight trials reported height[34 35 56-59 71 73] and all reported weight.[33-35 56-59 71-73] Four trials with individual patient data reported height[34 35 57 73] and five reported weight.[33-35 57 73] Together these trials included 1699 control and 2617 antibiotic treated participants, followed-up for a mean of 268 (SD 266) days, across seven countries. The mean age of participants ranged from 4 to 115 months (table 2). On average, trial participants were below the age standardised reference population mean for height or weight at baseline (table 1).

Risk of bias

Only one trial[59] was evaluated to be at high risk for bias overall (that is, when all bias domains were considered together). This was based on high risk as a result of inadequate random sequence generation, since treatment was randomly allocated to groups of children determined by the investigators; unclear risk due to inadequate allocation concealment; and high risk from differential attrition between treatment arms. Five trials were ranked as low risk for bias overall. These trials were low risk in all six bias domains.[33-35 57 73] Finally, four trials had an unclear risk for bias overall because the procedures were not fully described.[56 58 71 72]

For risk of bias due to attrition, the linear mixed models fit for the five trials with individual patient data were unbiased by losses to follow-up, provided the losses were uninformative conditional on observed height and weight. Among the trials with aggregate data only Guzman and colleagues[59] was determined as being impacted by drop outs. Gupta and colleagues[58] reported exclusions before randomisation but reported outcomes on all 79 participants recruited at baseline. Heikens and colleagues[56] reported that drop outs predominantly consisted of participants who moved too far from the study site to be followed-up or withdrew consent (9% and 2% of the total sample, respectively). Risk of bias due to attrition could not be assessed in the two remaining trials with aggregate data because the authors did not provide any data on exclusion of participants

during the study.[71 72] However, these trials only accounted for 5.9%[71] of the weights in the pooled treatment effect for height, and 1.2%[71] and 1.4%[72] in the pooled treatment effect for weight. Overall, we do not think that attrition posed a serious risk of bias in our analyses.

Egger's test suggested no significant publication bias among trials reporting height (P=0.841) or weight (P=0.391).

Meta-analysis

Our random effects models estimated an average treatment effect for height of 0.04 cm/month (95% confidence interval 0.00 to 0.07, fig 2⇓), and an average effect for weight of 23.8 g/month (95% confidence interval 4.3 to 43.3) in antibiotic treated compared with control children (fig 3⇓). The I^2 statistic showed a considerable degree of statistical heterogeneity in treatment effects for both height and weight (84.8% and 84.4%, respectively).

To assess the impact of antibiotic treatment on growth in children aged less than 2 years, we used the same two step approach described for the analysis using complete data. We fitted models of individual patient data restricted to participants less than 2 years old[33-35 57] and pooled these treatment effect estimates with the estimates for aggregated data reported by Heikens and colleagues,[56] which was the only trial with aggregated data restricted to this age group. These included observations from 833 control and 1461 treated infants, followed up for a mean 169 (SD 152) days. The treatment effect in these children was not statistically significant for height (0.03 cm/month, 95% confidence interval −0.05 to 0.11) but was for weight (29.6 g/month, 95% confidence interval 2.4 to 56.8), I^2=47.0%.

Metaregression analyses

Only geographical region significantly explained variation in the treatment effect across trials for weight (table 3⇓). The treatment effect was 35.6 g/month larger on average in trials conducted in Africa (95% confidence interval 12.8 to 58.3) compared with trials conducted in other regions. No statistically significant moderators of the height treatment effect were identified by bivariate analyses. We could not investigate risk of bias domains as moderators of treatment effect because only one trial was evaluated as high risk in any domain. All bivariate models included one treatment effect moderator and one outcome (table 3).

Duration of treatment, geographical region, treatment for *Giardia lamblia* infection, and age were statistically significant moderators of treatment effect, after adjustment for mean age of study population (table 4⇓). The height treatment effect was 0.001 cm/month (95% confidence interval −0.002 to 0.000) smaller on average with each one month increase in mean population age, and was 0.007 cm/month larger on average with each additional day of treatment (0.00 to 0.01). The weight treatment effect was 0.5 g/month smaller on average (95% confidence interval −1.0 to −0.1) with each one month increase in mean age, 33.2 g/month (5.3 to 61.2) larger on average in trials conducted in Africa, and 46.9 g/month (−83.2 to −10.6) smaller on average in trials in which participants were treated for *G lamblia* infection. In this last model, the intercept was 62.1 g/month (95% confidence interval 29.3 to 94.9), indicating a significant treatment effect in trials that did not treat children for *G lamblia* infection. All mean age adjusted models included mean participants' age, one treatment effect moderator, and one outcome (table 4).

Sensitivity analyses

Only removal of Prendergast and colleagues[35] from the random effects model impacted the average effect for height. Without this trial the average effect was 0.02 cm/month (95% confidence interval −0.01 to 0.05), a 50% decrease. The average treatment effect for weight was robust to the removal of trials. Also, two trials recruited children admitted to hospital.[33 72] Simultaneous exclusion of both trials did not change the average treatment effect for weight (21.5 g/month, 95% confidence interval 2.3 to 40.7). These two trials did not report height.

In addition we fit linear mixed models to investigate whether adjusting for participant age at the individual level (using trials with individual patient data only) would produce the same estimates of treatment effect moderation as we obtained by weighted metaregression adjusted for mean participant age (table 4). These models included age and duration of treatment, geographical region, or treatment for *G lamblia* infection, along with corresponding interaction terms. Results of these individual patient data models were consistent with the weighted metaregression results using all trials, with the exception of age, where the treatment effect on weight increased by 0.8 g/month for each one month increase in child age on average.

Subgroup analyses

The weight treatment effect was homogeneous across trials conducted in Africa using a random effects model (41.4 g/month, 95% confidence interval 31.0 to 51.7); I^2=0.0%. The average treatment effect estimated in this subgroup was identical when a fixed effects model was used (41.4 g/month, 31.0 to 51.7).

Discussion

In this pooled analysis of individual patient data and aggregate data from 10 randomised controlled trials conducted in seven low and middle income countries, antibiotic treatment had a positive average treatment effect on both height and weight in children aged 1 month to 12 years. Our results suggest that the growth promoting effect of antibiotics is more substantial for ponderal growth than for linear growth, and that the effect may be more homogenous in younger children. Analysis from the trials with individual patient data showed an increase in the weight treatment effect with increasing participants' age. This is in contrast with the results of the metaregression model, which suggested a smaller effect with increasing mean age. The trials with individual patient data primarily included children less than 5 years old, whereas two trials with aggregate data recruited older children.[51 79] Cross level bias may also partly explain this discrepancy. Although we did not restrict study selection to populations with a particular nutritional status, children were generally below the age standardised reference population mean for height or weight, reflecting the spectrum of stunting and wasting malnutrition seen in low and middle income countries. The larger weight treatment effects we observed in trials conducted in Africa may plausibly be explained by the high prevalence of HIV infection and severe acute malnutrition among populations included in these studies. Two trials conducted in Africa included severely malnourished children in whom all or a subset were infected with or exposed to HIV.[34 35] A third trial also included children from a similar high HIV prevalence community,[34 73] although HIV status was not specifically reported. The smaller weight treatment effect we observed in trials treating children for *G lamblia* infection suggested that growth may not be as strongly impacted in children treated with antibiotics for this specific protozoal

infection. Overall, the average treatment effects we observed would correspond to an approximate 0.1 increase in height for age Z score and a 0.2 to 0.3 increase in weight for age Z score over six months in HIV infected, HIV exposed, or severely malnourished populations under 2 years old using the World Health Organization growth standard.[74] These treatment effects therefore represent clinically relevant growth gains for the youngest children from the most vulnerable populations, in whom the long term impact of undernutrition is most profound.

Strengths and limitations of this study

The inclusion of trials with individual patient data and aggregate data served to improve the precision of our pooled estimates, minimised the risk of publication bias,[60] and allowed us to define height and weight in uniform units, avoiding the potential sources of bias inherent in utilising standardised mean differences.[75] We synthesised data from 4316 children, observed across a variety of antibiotics, indications for treatment, treatment regimens, and countries, providing the first comprehensive review of evidence from all randomised trials relating antibiotic use to growth in children in low and middle income countries, conducted over a 60 year period. A clear limitation of pooling such a diverse set of trials, with a large degree of statistical heterogeneity, is the limited generalisability of the average treatment effects. It is not completely clear which antibiotics or treatment regimens can be expected to produce these growth effects in other populations. However, pooling this diverse set of trials did allow identification of important subpopulations in whom the growth effect may be more profound when broad spectrum antibiotics are used. However, owing to the small number of trials, we had limited power to identify moderators of treatment effect, and we were not able to fully investigate trial level confounding with multivariable metaregression models. Specifically, the potential modifying effect of HIV prevalence, treatment duration, antibiotic class, concurrent nutritional interventions, and study population characteristics could not be fully elucidated. Also, cross level bias cannot be ruled out in our metaregression analyses of treatment effect moderators (which are measured at trial level); hence care must be taken in extending the treatment modifying effects to the individual level, particularly for age. Egger's test showed no evidence of publication bias. Careful screening of search results and communication with investigators ensured identification of published and unpublished reports. Finally, only one trial was evaluated to be at high risk for bias.[59]

Comparison with other studies

The exact reasons for the observed growth effects from antibiotics remain unclear, but several mechanisms may be involved. Respiratory and gastrointestinal infections are known to be associated with undernutrition. Malabsorption of nutrients, increased nutrient loss during episodes of diarrhoea, gut inflammation, impaired intestinal barrier function, diversion of nutrients away from growth to support immune activation, and loss of appetite are possible reasons for impaired growth during infection.[19-21] Antibiotics may improve growth by resolving subclinical and clinical infections. Eradication of microbes that regulate endocrine hunger signals may also contribute to growth gains with antibiotics. Changes in post-meal leptin and ghrelin serum levels, both of which help to regulate satiety, have been associated with the eradication of *Helicobacter pylori* following antibiotic treatment,[76] although this may play less of a role in food insecure settings.

An alternative possibility is that alteration of the intestinal microbiota by antibiotics may result in growth gains.[77-79] The intestinal microbiota regulates immune development and inflammation in the gut,[23 24] maintains host-microbe homeostasis in the gut,[25] and has an important role in nutrient harvesting and absorption.[26] Disturbance of intestinal microbiota composition resulting from chronic intestinal colonisation with pathogens or overgrowth of commensal bacteria in the small intestine[19 27-29] may lead to disruption of these functions. Perturbation of the intestinal microbiota may also lead to intestinal inflammation and increased intestinal permeability. These changes are characteristic of environmental enteropathy, a subclinical disorder of the intestinal tract that is ubiquitous in developing countries and is associated with poor linear growth.[19 27-29]

Antibiotics are known to induce changes in the composition of microbiota in the gut,[30 31] and these changes may persist.[77 79] Recent work has shown that intestinal microbial taxa may not return to their pretreatment abundance levels, even after a single use of antibiotics[77 79 80]; however, the extent of recovery to baseline may depend on the class of antibiotic used.[79] A recent review qualitatively summarised the evidence supporting a relation between antibiotic use and weight and included evidence from some trials in humans.[77] The mechanisms underlying these growth benefits plausibly include resolution of underlying infections or inflammatory processes (for example, environmental enteropathy) or alteration of intestinal microbiota composition and function. In an experimental animal model, weight loss in mice resulted from transplantation of donor faeces from children with kwashiorkor, but not from their healthy twins,[81] whereas increases in total body mass and fat mass were induced in mice transplanted with donor faeces from obese adults, but not from their lean twins.[82] Although we cannot rule out an effect of antibiotics on latent bacterial infections in the included trials, it is plausible that the growth benefits we observed also encompass an important growth effect mediated by intestinal microbiota.

Conclusions and policy implications

In summary, our results show that antibiotic treatment has a growth promoting effect, particularly for ponderal growth, in prepubertal children from undernourished populations in low and middle income countries. Linear growth seems less responsive to antibiotics. A better understanding of the biological mechanisms behind these antibiotic associated effects on growth is critical for certain populations, such as children under 2 years old (as reversal of stunting beyond this age is challenging[2]), and HIV infected, HIV exposed, and acutely malnourished children in whom antibiotics continue to be a standard component of care.[36 83 84] Antibiotics, however, are not the most viable option for the treatment of malnutrition outside of these highly vulnerable populations in which antibiotic treatment is already routinely recommended for treatment and prevention of infections. In addition to concerns about antimicrobial resistance, antibiotic use has also been associated with adverse events such as antibiotic associated diarrhoea. The growth benefits of more widespread antibiotic use may not outweigh the risks. Our findings highlight the co-benefits of antimicrobial treatment that have been previously reported from developing countries[34 35] and provide an intriguing proof of concept that treatment of subclinical infections and modulation of the intestinal microbiota may have beneficial effects on growth.

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What is already known on this topic

Antibiotics have shown variable effects on growth in humans but are currently recommended for severely malnourished children and those infected with or exposed to HIV to reduce morbidity and mortality

Several mechanisms exist through which antibiotic treatment may affect growth in children, including resolution of infection and, potentially, alteration of the intestinal microbiota

What this study adds

Evidence from a diverse set of randomised controlled trials show that antibiotic use in prepubertal children from undernourished populations in low and middle income countries leads to clinically relevant growth gains, particularly for weight

Larger growth gains are associated with antibiotic use in studies with a high prevalence of HIV infection and severe acute malnutrition

The growth gains show the co-benefits of antibiotic treatment in high risk populations, and provide proof of concept that treatment of infections or modulation of the intestinal microbiota can have beneficial growth effects; however, more research is needed to better understand the mechanisms involved

Contributors: EKG, EEEM, JHH, RJS, and ARM created and designed the study. EKG and SMAJ conducted the literature search, study selection, and data collection. AJP adjudicated study selection. AJP, DMG, ASW, IT, RG, ST, and MBdM provided individual patient data, and provided insight into trial design, implementation, and database structure. EKG did the data analysis. EKG, EEEM, and ARM interpreted the data and drafted the manuscript. All authors critically revised the manuscript for intellectual content, discussion of findings, and overall conclusions. ARM is the guarantor.

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Transparency: ARM affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Data sharing: The electronic data abstraction form is available from the first author at ethan.gough@mail.mcgill.ca.

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Tables

Table 1| Characteristics of randomised controlled trials of antibiotic use and growth in prepubertal children included in meta-analysis

Study, country	Indication for treatment	Eligibility criteria	Baseline nutritional status	Intervention		
				Antibiotic	Control	Concurrent
Scrimshaw et al 1953[71], Guatemala	Malnutrition	Schoolchildren	Children in participating communities averaged 2-4 years below US reference for height and weight	Aureomycin	Placebo	Enriched soya milk powder given 6 days/week except during holidays
Guzman et al 1958[59], Guatemala	Malnutrition	Schoolchildren	Children in participating communities averaged 2-4 years below US reference for height and weight	Aureomycin or penicillin	Placebo	None
Wolfsdorf et al 1973[72], South Africa	Diarrhoea with or without vomiting	Infants presenting with diarrhoea or vomiting severe enough to warrant hospital stay	Not recorded	Trimethoprim-sulphonamide	Placebo	"Routine" treatment regimens carried out
Gupta et al 1982[58], Guatemala	*Giardia lamblia*	Children	Mean percentage height and weight for age: 88.6% and 71.5%	Metronidazole	Placebo	None
Heikens et al 1993[56], Jamaica	Malnutrition	Children malnourished according to Wellcome classification, excluding children with oedema, congenital abnormality, infection requiring hospital stay, or anorexia preventing normal home feeding	Mean percentage height and weight for age: 88.6% and 65.1%	Metronidazole	None	Multivitamins and folic acid, outpatient treatment of infection or illness, advice on breast feeding and weaning for duration of follow-up
Tahan et al 2007[33], Brazil	Diarrhoea	Infants with diarrhoea for at least 7 days who needed hospital stay, excluding infants with associated disorders, use of antibiotics in preceding 7 days, or evidence of systemic infection	Mean height and weight for age Z scores: −2.02 and −2.36	Polymixin B	Placebo	None
Goto et al 2009[57], Bangladesh	*G lamblia*	Infants	Mean height and weight for age Z scores: −1.05 and −1.82	Secnidazole	Placebo	None
Trehan et al 2009[73], Malawi	Environmental enteropathy	Children, excluding those with chronic debilitating illnesses or evidence of severe acute malnutrition	Mean height and weight for age Z scores: −1.67 and −0.91	Rifaximin	Placebo	None
Prendergast et al 2011[35], Zambia	Prophylaxis against opportunistic infection	Children with positive HIV antibody test result, excluding those with opportunistic infection, life expectancy ≤4 weeks, current cotrimoxazole treatment or allergy to this drug, or previous *Pneumocystis jirovecii* pneumonia	Mean height and weight for age Z scores: −3.55 and −3.10	Cotrimoxazole	Placebo	None
Trehan et al 2013[34], Malawi	Severe acute malnutrition	Children with oedema, or weight for height Z score ≤3	Mean height for age Z score was −3.19	Amoxicillin or cefdinir	Placebo	Standardised nutrition counselling and ready to use therapeutic food at dose of approximately 175 kcal/kg/day given in 2 week intervals

Table 2| **Growth outcomes and potential treatment effect moderators in randomised controlled trials of antibiotic use and growth in prepubertal children included in meta-analysis**

				Antibiotics					Mean growth/month of follow-up			
									Height (cm)		Weight (g)	
Study	IPD	Mean (SD) age (months)	No (%) male	Class, spectrum	Dosage	Doses/day	Days treated	Mean follow-up (days)	Controls	Treated	Controls	Treated
Scrimshaw et al[71]	No	114.9 (NR*)	143 (57.2)*	Bacteriostatic, broad spectrum	50 mg	1	667†	758	0.39	0.42	180.0	270.0
Guzman et al[59]	No	114.9 (NR)	143 (57.2)	Bacteriostatic, broad spectrum	50 mg	1	394†	394	0.36	0.36	170.0	166.0
Wolfsdorf et al[72]	No	5.9 (6.4)	NR	Bactericidal, broad spectrum	NR	NR	NR	91	NR	NR	664.0	788.4
Gupta et al[58]	No	23.0 (17.2‡)	NR	Bactericidal, narrow spectrum	25 mg/kg	2	42	NR	0.51	0.58	135.9	154.2
Heikens et al[56]	No	14.1 (6.5)	NR	Bactericidal, narrow spectrum	20 mg/kg	1	5	179	12.40	12.20	1336.7	1393.3
Tahan et al[33]	Yes	4.0 (2.0)	17 (68.0)	Bactericidal, narrow spectrum	2.5 mg/kg	4	7	7	NR	NR	710.5§	735.7§
Goto et al[57]	Yes	8.6 (3.2)	135 (50.4)	Bactericidal, narrow spectrum	35 mg/kg	1	10	264	9.11	9.12	1105.1	1100.7
Trehan et al[73]	Yes	47.2 (7.12)	60 (41.7)	Bacteriostatic, broad spectrum	10 mg	2	7	28	107.56¶	107.96¶	14 957.1¶	15 030.3¶
Prendergast et al[35]	Yes	64.5 (44.7)	266 (49.2)	Bactericidal, broad spectrum	240 g (<5 yrs); 480 g (>5 yrs)	1	575	575	5.66	5.77	803.9	845.0
Trehan et al[34]	Yes	21.1 (9.1)	1317 (47.6)	Bactericidal, broad spectrum	7 mg/kg (cefdinir); 40-45 mg/kg (amoxicillin)	2	7	43	26.74	26.76	2898.0	2938.9

IPD=individual patient data; NR=not reported.

*Not reported by Schrimshaw et al.[71] Values assumed to be same as in Guzman et al[59] as both studies were conducted in communities in Guatemalan highlands in 1950s by same research group and recruited children in 5-12 year age range.

†Estimated from mean number of treatment days reported per trial arm.

‡Not reported by Gupta et al,[58] estimated from Schrimshaw et al 1968.[85]

§Mean change in weight per day; follow-up was seven days.

¶Follow-up was 28 days; these represent height and weight at end of follow-up.

Table 3| **Estimated average differences in antibiotic treatment effects on growth in prepubertal children, using weighted bivariate random effects metaregression**

	Height (cm/month)				Weight (g/month)			
Trial characteristics	**No**	**Mean difference**	**P value**	**I^2 (%) (95% CI)**	**No**	**Mean difference**	**P value**	**I^2 (%) (95% CI)**
Geographical region (Africa *v* other)	8	0.05	0.275	79.6 (39.7 to 98.8)	10	35.57	0.002	50.9 (10.6 to 99.1)
Publication year	8	0.00	0.650	78.7 (35.5 to 99.0)	10	0.50	0.275	77.6 (47.1 to 99.9)
Treatment effect adjusted for baseline imbalances (yes *v* no)	8	0.00	0.964	88.5 (55.9 to 99.9)	10	−17.59	0.465	85.4 (62.8 to 99.9)
Mean length of follow-up (days)	7	0.00	0.282	79.8 (35.3 to 99.4)	9	−0.05	0.490	87.5 (61.1 to 99.9)
No of doses/day	8	0.02	0.648	83.4 (44.1 to 98.8)	9	21.70	0.307	84.4 (56.9 to 99.7)
Duration of treatment (days)	8	0.00	0.340	81.9 (47.1 to 99.1)	9	0.00	0.921	86.6 (65.6 to 99.9)
Antibiotic class (bactericidal *v* bacteriostatic)*	7	−0.05	0.792	71.6 (25.9 to 99.6)	8	−51.61	0.727	87.1 (72.1 to 100.0)
Antibiotic spectrum (broad *v* narrow)	8	0.02	0.666	89.2 (57.2 to 99.3)	10	9.41	0.666	84.6 (61.0 to 99.9)
Participants given concurrent nutritional intervention (yes *v* no)	8	−0.05	0.356	82.5 (44.9 to 98.6)	10	31.00	0.110	75.7 (44.2 to 99.9)
Mean age (months)	8	0.00	0.948	82.0 (1.7 to 96.7)	10	−0.24	0.381	82.0 (54.3 to 99.9)
Treatment was for malnutrition (yes *v* no)	8	−0.06	0.066	75.2 (17.4 to 99.0)	10	2.65	0.906	85.1 (62.5 to 99.9)
Treatment was for *Giardia lamblia* infection (yes *v* no)	8	0.01	0.833	88.6 (56.2 to 99.4)	10	−26.42	0.210	82.2 (55.1 to 99.9)
Treatment was for diarrhoea with or without vomiting (yes *v* no)†	NA	NA	NA	NA	10	144.37	0.075	85.3 (60.9 to 99.8)

NA=not applicable.

*Excludes Prendergast et al[35] and Wolfsdorf et al[72] as not clear whether trimethoprim with sulphonamide or sulfamethoxazole are bacteriostatic or bactericidal in combination.

†No trials reporting height treated participants for diarrhoea with or without vomiting.

Table 4| **Significant moderators of antibiotic treatment effects on growth in prepubertal children, using weighted random effects metaregression adjusted for mean study population age**

Trial characteristics	No	Mean difference (95% CI)	I^2 (%) (95% CI)
Height model 1 (cm/month):			
Duration of treatment (days)	8	0.007 (0.00 to 0.01)	53.6 (0.0 to 99.3)
Mean age (months)	8	−0.001 (−0.002 to 0.00)	
Weight model 1 (g/month):			
Geographical region (Africa *v* other)	10	33.2 (5.3 to 61.2)	53.5 (3.6 to 99.9)
Mean age (months)	10	−0.2 (−0.4 to −0.1)	
Weight model 2 (g/month)			
Treatment was for *Giardia lamblia* (yes *v* no)	10	−46.9 (−83.2 to −10.6)	57.8 (9.3 to 99.9)
Mean age (months)	10	−0.5 (−1.0 to −0.1)	

Figures

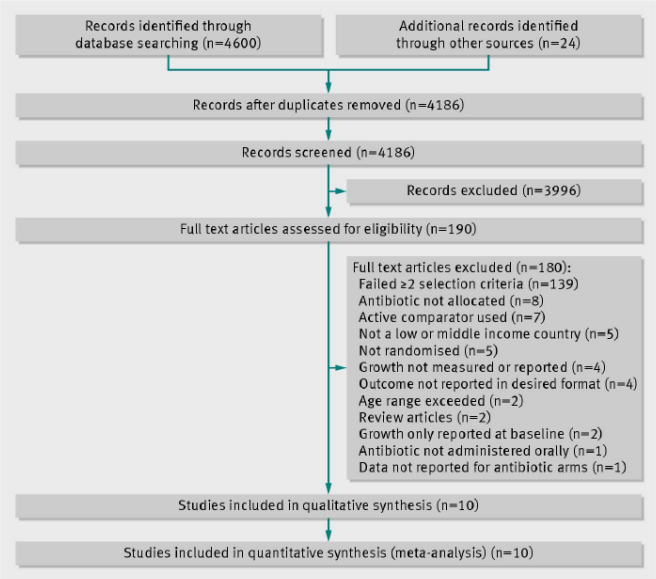

Fig 1 Flow diagram of search retrieval and trial selection

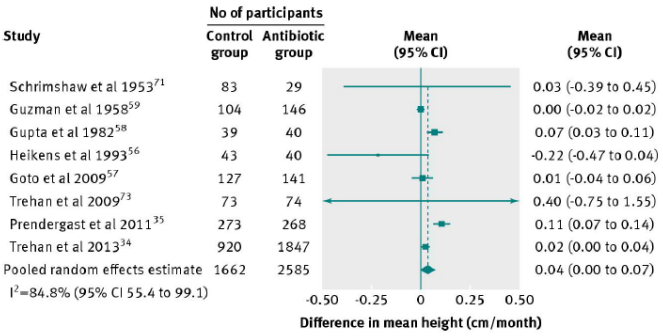

Fig 2 Random effects meta-analyses and forest plots of antibiotic use and height. Point size reflects study weight

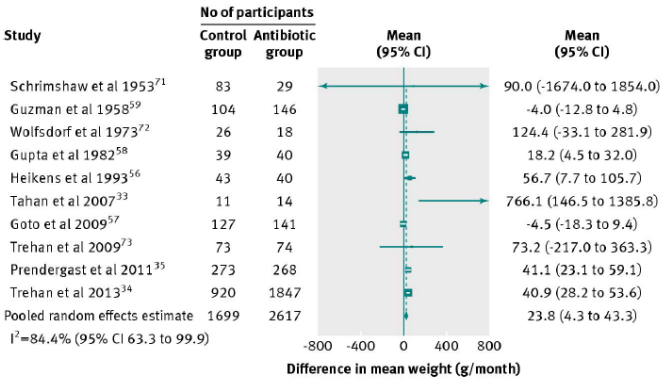

Fig 3 Random effects meta-analyses and forest plots of antibiotic use and weight. Point size reflects study weight

12. Linear growth faltering in infants is associated with *Acidaminococcus sp*. and community-level changes in the gut microbiota

12.1. PREFACE TO "LINEAR GROWTH FALTERING IN INFANTS IS ASSOCIATED WITH ACIDAMINOCOCCUS SP. AND COMMUNITY-LEVEL CHANGES IN THE GUT MICROBIOTA"

The ecosystem of microbes in the human gut performs many important functions in immune system regulation and defense; nutrient harvesting and absorption. It has long been proposed that the intestinal microbiota may play an important role in child malnutrition. Recent advances in technology have allowed investigation and characterisation of the intestinal microbiota on an unprecedented scale. Animal models provide very convincing evidence that the composition of this ecosystem, in terms of the types and abundances of bacteria that are present, can induce weight gain or loss. However, the specific characteristics of the gut microbiota that cause these changes in growth remain unknown, and no studies to date have investigated the microbiota as a determinant of linear growth. In this manuscript, I investigate differences in the structure of gut microbiota communities in stunted infants, and identify changes in gut microbiota composition that are temporally associated with deficits in linear infant growth. These results provide the first evidence that the gut microbiota may be an important unrecognized factor in linear growth faltering, and provide a biologically plausible mechanism through which growth deficits may occur.

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12.2. MANUSCRIPT: "LINEAR GROWTH FALTERING IN INFANTS IS ASSOCIATED WITH ACIDAMINOCOCCUS SP. AND COMMUNITY-LEVEL CHANGES IN THE GUT MICROBIOTA"

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Abstract

Background: Chronic malnutrition, termed stunting, is defined as suboptimal linear growth, affects one-third of children in developing countries, and leads to increased mortality and poor developmental outcomes. The causes of childhood stunting are unknown and strategies to improve growth and related outcomes in children have only had modest impacts. Recent studies have shown that the ecosystem of microbes in the human gut, termed the microbiota, can induce changes in weight. However, the specific changes in the gut microbiota that contribute to growth remain unknown, and no studies have investigated the gut microbiota as a determinant of chronic malnutrition. **Results:** We performed secondary analyses of data from two well-characterized twin cohorts of children from Malawi and Bangladesh to identify bacterial genera associated with linear growth. In a case-control analysis, we used the graphical lasso to estimate covariance network models of gut microbial interactions from relative genus

abundances, and used network analysis methods to select genera associated with stunting severity. In longitudinal analyses, we determined associations between these selected microbes and linear growth using between-within twin regression models to adjust for confounding and introduce temporality. Reduced microbiota diversity and increased covariance network density were associated with stunting severity; while increased relative abundance of *Acidaminococcus sp*. was associated with future linear growth deficits. **Conclusions:** We show that length growth in children is associated with community-wide changes in the gut microbiota and with the abundance of the bacterial genus, Acidaminococcus. Larger cohorts are needed to confirm these findings and to clarify the mechanisms involved.

Introduction

Undernutrition in early childhood underlies 45% of mortality in children aged under 5 years worldwide, resulting in 3.1 million deaths annually (1). Ponderal and linear growth faltering in children are viewed as indicators of acute and chronic malnutrition, respectively, and are often measured in terms of z-scores (i.e. deviations in attained growth from a reference population mean). Children whose length- or height-for-age z-scores (LAZ or HAZ) is more than 2 standard deviations below the reference population mean are termed stunted. Stunting has short-term effects on morbidity and mortality (3), leads to poor motor development and cognition, and reduces educational and economic attainment over the life-course (1,3,28). An estimated 165 million children under 5 years old were stunted in 2011 (1), representing almost one-third of children in this age group in low- and middle-income countries (LMICs), hindering developmental potential and human capital of entire societies.

Most linear growth faltering occurs in the period from conception to 2 years of age, and restoration of deficits in linear growth beyond that period is limited. Interventions to prevent stunting are therefore required early in the life-course. Social, economic, and educational factors, as well as infectious diseases and poor diet in early childhood all contribute to linear growth faltering (1,77–80). Furthermore, a number of studies have shown that small intestinal inflammation and permeability are associated with poor linear growth (86–88,210). This sub-

clinical gut pathology has been termed environmental enteric dysfunction (EED), and is acquired early in life among children living in unsanitary conditions (78,90–93). Reduced intestinal barrier function caused by EED enables bacterial translocation to occur, leading to chronic systemic inflammation, which is associated with reduced insulin-like growth factor 1 (IGF-1) and linear growth faltering (96). However, the pathophysiology of stunting is not well understood and currently available interventions, which focus mostly on dietary supplementation and prevention of diarrhea, have only a modest impact (6). Mechanisms underlying stunting therefore need to be better defined so that tractable pathways for intervention can be identified.

Recent studies suggest a role of the intestinal microbiota in child growth. The intestinal microbiota is an ecosystem of gut microbes that helps to modulate nutrient harvesting from the diet, mucosal inflammation, and the immune response in the gut (9,10,126,128,129). Observational studies in humans (130–133) have demonstrated a relationship between the intestinal microbiota and severe acute malnutrition (SAM). A causal effect of the intestinal microbiota on weight has also been shown using experimental animal models (14,15). However, the specific changes in the microbiota that contribute to growth remain unclear, and no studies to date have investigated the intestinal microbiota as a determinant of linear growth.

We performed a secondary analysis of publicly available data from two twin cohorts of undernourished children from low-income settings (Malawi and Bangladesh) (14,133), to identify bacterial genera whose relative abundances explain linear growth. Previous analyses from these cohorts showed that acute malnutrition was associated with differences in gut microbiota functional gene abundances (14) and maturation (133). Our analyses aimed to determine changes in gut microbiota networks and relative abundance associated with stunting status, in order to identify potential microbiota members that contribute to linear growth faltering (i.e. chronic malnutrition). We hypothesized that differences in the relative abundance

of identified genera are independently associated with prospective deficits in linear growth between siblings.

Methods

Study Sample

Demographic, clinical and anthropometric data from a cohort of 22 twin pairs from Malawi, and a second cohort of 11 twin pairs plus one set of triplets from Bangladesh, were made available at http://gordonlab.wustl.edu/SuppData.html. Details are provided in Smith *el al*. (14) and Subramanian *et al*. (133). In brief, 22 twin pairs ages birth to 3 years were selected from among 317 available pairs in five rural communities in Malawi for longitudinal analyses of their gut microbiotas. Twin pairs were selected if at least four fecal samples were available from each co-sibling. The 12 sets of siblings from Bangladesh were selected from among mothers with multiple pregnancies at a child health and family planning clinic in Dhaka, and were followed up for longitudinal gut microbiota evaluation. In both twin cohorts, at each follow-up visit length/height and weight were measured, and fecal samples were collected along with data on age in months, and diarrhea in the 7 days prior to or at the visit for Malawi and Bangladesh, respectively. Anthropometric measures were provided as height-for-age and weight-for-height z-scores. In the Malawi cohort, if at least one co-twin developed SAM, as defined using WHO criteria (134), both were treated with ready-to-use therapeutic food (RUTF).

Whole Genome Sequencing and Annotation

Whole genome sequence datasets from the Malawi cohort were made available through the European Bioinformatics Institute at http://www.ebi.ac.uk/ena/data/view/ERP001911&display=html (14), and MG-RAST (http://metagenomics.anl.gov/) (138). Relative genus abundances (expressed as a percentage of the total amount of DNA assigned to a bacterial taxon in each stool sample) were estimated from shotgun reads using MetaPhlan (253). Relative Operational Taxonomic Unit (OTU) abundance data from the Bangladesh cohort were used as provided at http://gordonlab.wustl.edu/SuppData.html, and were analysed at the genus level. Extraction of

genomic DNA from fecal samples; DNA sequencing; processing and filtering of reads; and, for Bangladesh data, OTU picking and taxon assignment have been described (14,133). The Simpson diversity index was calculated as a measure of alpha diversity in all samples using *vegan* (254). Simpson diversity measures the probability that two randomly selected microbes in a sample will be from different taxa, and provides a measure of the number of different types of bacteria present.

Statistical Analyses

Analyses were performed separately for the Malawi and Bangladesh cohorts using two approaches. We first conducted an analysis of unmatched cases and controls selected from each cohort in order to identify changes in microbiota networks and relative genus abundance associated with stunting status, and to select genera for inclusion in longitudinal analyses. Next, in longitudinal analyses, we fitted multivariable regression models, using data available at all follow-up visits for the entire cohort of children, to control for confounding and to introduce temporality.

Case-Control Network Analyses. Children in the Malawi and Bangladesh twin cohorts had median baseline HAZ of -2.96(IQR:-3.68,-2.18) and -3.75(IQR:-4.54,-2.68) respectively, indicating that the majority were severely stunted at study entry (*Appendix 7*). For the case-control analyses, linear growth status was therefore defined as severely stunted (HAZ≤-3, cases) or stunted (-3<HAZ≤-2, controls). For cases, the visit where a child first reached HAZ≤-3 was selected, excluding children already severely stunted at study entry. The subset of children who were not siblings of cases, and who had HAZ>-3 but ≤-2 at the end of follow-up, regardless of their baseline z-score were selected as controls. Where both siblings in a twin pair met case or control criteria, one was randomly chosen to avoid within-group correlations (255), and data from only one visit were used per child. Differences in anthropometric, demographic and epidemiological measures, alpha diversity, and relative abundance between cases and controls were evaluated using the Chi-squared test or by permutation test on the median, as appropriate.

A supplemental approach to diversity indices for investigating the microbiota uses networks of pairwise correlations between taxa as a model of microbe-microbe interactions. In this representation, nodes are genera and a link between two nodes represents a non-zero association between two genera. This association is used as a proxy for bacterial interaction (see *Appendix 1 for further information*). An alternative to pairwise correlations is to estimate an inverse covariance matrix from genus abundances as a graphical model of important bacterial relationships. We generated these graphical models separately for cases and controls using the graphical lasso (glasso) (256). The covariance associations estimated by the glasso (i.e. the links between genera in each network) are independent of all other taxa and covariates included in the model. For each case and control network, we calculated graph density, and the normalized degree centrality of each genus (257) using *igraph* (258). Differences in network indices were assessed for statistical significance by permutation. Specifically, children were randomly reallocated between the case and control groups 1000 times. For each permutation, one network was estimated per group and distributions of the difference in network indices between case and control networks were generated for statistical inference. Genera with significant differences in degree centrality or relative abundance between cases and controls were selected for longitudinal analyses.

Longitudinal Analyses. After performing microbiota feature selection in the case-control analyses, we fitted between-within regression models (259,260), using data for all follow-up visits from all twin pairs in each cohort (regardless of their selection as cases or controls), to investigate whether the relative abundance of selected genera was associated with linear growth. A between-within model allows estimation of the effect that differences in exposure level (e.g. differences in genus abundance) between siblings within a twin pair have on their outcomes, while adjusting for unmeasured confounders that siblings share, such as fetal, maternal, and environmental factors. This is done by including both (i) individual sibling exposure values and (ii) the mean exposure value of co-twins as covariates in a regression

model. Adjustment for measured confounders not shared between co-twins (e.g. diarrhea) can be made by including sibling-specific covariates in the model (260).

We fitted a separate model for each genus selected, with relative abundance as the exposure and HAZ as the outcome. Each model was adjusted for reported diarrhea, WHZ, and alpha diversity as reported confounders not shared by co-twins. Age in months and length of follow-up since baseline were also included as predictors of the outcome. All covariates were lagged by one visit in order to model their effect on future HAZ, with the exception of length of follow-up and age. All between-within models were fitted by Generalized Estimating Equations (GEE) using *geepack* (262), and multiple hypothesis testing adjustments using the Benjamini-Hochberg method (263) were made. Statistical significance was determined at $\alpha<0.1$ due to the small sample size of both cohorts. All analyses were conducted in *R* version 3.0.1.

Results and discussion

Cohort Description

Data were provided for 44 children in the Malawi cohort, who were median 10.2 months (IQR:4.6,14.5) old at baseline and followed for median 9.7 months (IQR:4.1,14.5). Baseline HAZ and weight-for-height z-scores (WHZ) were -2.95(IQR:-3.70,-2.18) and -0.46(IQR:-0.87,-0.13), respectively. Anthropometric, epidemiological and DNA whole genome shotgun sequencing data were provided for median 7(IQR:4,8) follow-up visits per child, for a total of 308 longitudinal observations (*Appendix 2*). Data were available for 25 children in the Bangladesh birth cohort, who were 0.3 months (IQR:0.19, 0.63) old at baseline and followed for median 14.5 months (IQR:11.9,20.7). Baseline HAZ and WHZ were -3.75(IQR:-4.54,-2.68) and -0.57(IQR:-0.51,0.35) respectively. Anthropometric, epidemiological and relative abundance data were provided for median 17(IQR:13,22) follow-up visits per child. Randomly excluding one child from the set of triplets for between-within regression analyses provided 429 longitudinal observations.

Description of Cases and Controls

In the Malawi cohort, 13 children had a follow-up visit that met incident case criteria for severe stunting, and eleven had a follow-up visit that met control criteria for stunting (see Methods for details on case and control definitions). Six eligible cases were co-twins, and six eligible controls were also co-twins. In the Bangladesh cohort, 8 children had a follow-up visit that met incident case criteria, and 10 had a follow-up visit that met control criteria. Four eligible cases were co-twins, and 10 eligible controls were co-twins. For each pair of co-twins that both met case criteria, we randomly chose one sibling as a case to avoid within-group correlations (255). The same was done for pairs of co-twins that both met control criteria. This provided 10 cases and 8 controls from Malawi, and 6 cases and 5 controls from Bangladesh (*Figure 1*). Cases from the Malawi cohort had lower HAZ (-3.08 v -2.45, p<0.01), and were younger compared to controls (10.8 months v 19.6 months, p=0.05). Similarly, in the Bangladesh cohort, case HAZ was -3.17 v -2.63 for controls, p<0.01, and age was 2.9 v 11.0 months, p<0.01. WHZ was also higher in Bangladesh cases compared to controls (0.53 v -0.64, p=0.05) (*Appendix 2*).

Genus Relative Abundance and Microbiota Diversity

Roche 454 shotgun whole genome sequence data were provided for median 76,700(IQR:55,200, 103,000) reads per sample in the entire Malawi cohort, while relative abundance data from the Bangladesh cohort were quantified from a median 20,192(IQR:16,155, 24,632) reads. In both cohorts, a similar number of reads were available for cases and controls (*Appendix 2*).

In the Malawi cohort, Bifidobacterium (42.8%) and Prevotella (22.7%) were the most abundant genera identified, followed by Bacteroides (3.7%), Faecalibacterium (3.14%), Collinsella (1.0%), Lactobacillus (0.6%), and Blautia (0.6%). In the Bangladesh cohort, Bifidobacterium (46.2%), Streptococcus (4.8%), Lactobacillus (2.6%) and Escherichia/Shigella (1.8%) were the most abundant genera, followed by Collinsella (0.5%). These were also the most prevalent genera identified in fecal samples collected during follow-up (*Appendix 3*), and are consistent with the literature on microbiotas in infants and with different diets (264–269). In the Malawi cohort, Prevotella (18.1 v 42.9), Bacteroides (1.9 v 7.4), Eubacterium (0.0 v 2.4), and Blautia (0.6 v 2.4)

showed the largest decrease in relative abundance in cases v controls (*Appendix 4*). In the Bangladesh cohort, Lactobacillus (0.1 v 8.7), Olsenella (0.0 v 0.8), Dorea (0.0 v 0.7), Blautia (0.0 v 0.2), and unclassified genera in the Coriobacteriaceae (0.0 v 0.3) and Enterococcaceae (0.0 v 0.1) families showed the largest decrease in relative abundance in cases v controls. Lesser, but statistically significant depletion of Anaerococcus, Dialister, Faecalibacterium, Megamonas, Weissella, Megasphaera, and unclassified genera in the Lachnospiraceae, Lactobacillacaea and Veillonellaceae families were also observed in Bangladesh cases, while Bacteroides (0.5 v 0.0) was enriched (*Appendix 5*). Case microbiota were less diverse than controls in both cohorts (Malawi: 0.5 v 0.7, p=0.02; Bangladesh: 0.5 v 0.7,p=0.05) (*Appendix 2*).

Network Indices

Network density (i.e. the probability that two randomly selected microbes co-vary) was greater in case compared to control networks in both cohorts (Malawi: 0.56 v 0.25, p=0.08; Bangladesh: 0.56 v 0.33, p=0.42), indicating a greater potential for information flow in case microbiotas. We also observed that the density of edges from aerobes to anaerobes was greater in the case network in both populations (*Figures 2 & 3*).

In the Malawi cohort, differences in degree centrality were observed for Acidaminococcus (0.6 v 0.0), Bacteroides (0.6, v 0.2), Brachyspira (0.6, v 0.0), Haemophilus (0.6 v 0.2) and unclassified genera in the Neisseriaceae (0.6 v 0.2) and Chlamydiaceae (0.6 v 0.0) families in case v control networks (*Appendix 4*). In the Bangladesh cohort, Acinetobacter (0.5 v 0.0), Anaerococcus (0.7 v 0.2), Blautia (0.7 v 0.2), Coprococcus (0.5 v 0.0), Geobacillus (0.6 v 0.0), Lactococcus (0.6 v 0.0), Micrococcus (0.5 v 0.0), Proteus (0.6 v 0.0), and Sarcina (0.6 v 0.0) were more central in the case network (*Appendix 5*).

Between-Within Models

Thirty of 164 genera identified across both populations were selected, based on statistically significant differences in relative abundance or centrality, to estimate their association with future HAZ using multivariable between-within regression models. Acidaminococcus, of the

phylum Firmicutes, was the only genus associated with HAZ in longitudinal analyses of both cohorts. In the Malawi cohort, a 0.1% difference in the relative abundance of this genus between co-twins, was associated with a 0.08 lower height-for-age z-score (90%CI:-0.12,-0.04) at the subsequent study visit in the co-twin who had the greater Acidaminococcus abundance compared to their sibling. In the Bangladesh cohort, a 0.1% difference in the relative abundance of this genus between co-twins, was associated with a 0.19 lower HAZ (90%CI:-0.25,-0.13) at the subsequent visit in the co-twin with the greater Acidaminococcus abundance. These associations remained significant after controlling for multiple hypothesis testing (*Table 1*).

The literature on *Acidaminococcus sp,* with which we can infer its role in the human gut and its potential impact on linear growth in children, is sparse. Only two species in this genus have been described (270,271). One notable characteristic of these described species is their ability to consume glutamate as their sole source of carbon and energy. In porcine models, dietary glutamate is an essential oxidative fuel for the intestinal epithelium (272,273), which undergoes a continuous process of regeneration and has high energy demands. Estimates for the amount of glutamate completely metabolized in the gut range from 64% (273) to 90% (272). As such, glutamate is important to gut epithelium restitution. The beneficial effect of glutamate on restoration of gut barrier function has been observed using in vitro cell lines (274–276), as well as in animal models of glutamate supplementation (277–280). Glutamate is an important precursor and intermediate in the synthesis and metabolic recycling of other amino acids, and with the urea cycle, in the gut (272,273,281,282). Amino acids closely interlinked with glutamate metabolism include arginine, which also contributes to epithelium restitution, preserves barrier function, prevents accumulation of ammonia in the gut, and attenuates intestinal tissue damage (252–254); and glutathione, which protects the epithelium from damage by oxidative stress (283,284). Altogether, major functions of glutamate in the gut appear to be its role as a key intermediate in gut amino acid metabolism and nitrogen cycling; maintenance of epithelial integrity; and preservation of barrier function. Biomarkers of intestinal injury and repair have been associated with lower HAZ in LMICs (285). Impaired gut

barrier function is characteristic of EED, which is also associated with poor linear growth (86–88,210).

This evidence led us to pose the *a posteriori* hypothesis that glutamate fermentation by microbes is negatively associated with future HAZ. We tested this hypothesis using KEGG enzyme abundance data provided for the Malawi cohort. We fitted between-within regression models where the relative abundance of critical genes utilized in glutamate fermentation pathways by microbes (286) was the exposures of interest. We found that the abundance of genes encoding glutamate dehydrogenase and α-keto-glutarate reductase were negatively associated with future HAZ. For glutamate dehydrogenase and α-keto-glutarate reductase respectively, a one unit greater gene abundance in one co-twin compared to their sibling was associated with a -0.17(90%CI:-0.29,-0.04, p=0.03) and -0.08(90%CI:-0.16,-0.01,p=0.07) smaller HAZ in that co-twin at the subsequent study visit. These are the first two enzymes involved in the hydroxyglutarate fermentation pathway used by *Acidaminococcus fermentans* for glutamate fermentation; some species in the Peptoniphilus, Fusobacterium, and Clostridia families can also utilize this pathway (286,287).

In the Bangladesh cohort, we also observed a -0.003(90%CI:-0.004,-0.002) lower HAZ and a 0.001(90%CI:0.000,0.001) greater HAZ at the subsequent visit in co-twins who had a 0.1% greater abundance of Weissella or Blautia, respectively, compared to their siblings (*Table 1 & Appendix 6*). The association with Blautia was not statistically significant after controlling for multiple hypothesis testing.

Discussion

In these analyses, we show that less diverse gut microbiotas with greater covariance network density are associated with stunting severity, and an increase in the relative abundance of *Acidaminococcus sp* is associated with lower future linear growth in two very different, well characterized cohorts of children living in low-income settings. We applied a novel approach, utilising a statistical learning method combined with network analysis and a permutation test to

determine differences between microbiota communities of stunted and severely stunted children from these cohorts, and applied longitudinal epidemiological analysis methods to investigate whether changes in the genera identified were associated with future linear growth.

In our longitudinal models, greater abundance of Acidaminococcus was associated with a future deficit in HAZ between co-twins in both cohorts. *Acidaminococcus sp.* can utilize glutamate as their sole source of carbon and energy. Greater abundance of genes encoding the first two enzymes in the hydroxyglutarate pathway for glutamate fermentation was also associated with a future HAZ deficit. Overgrowth of bacteria that can ferment glutamate may have a deleterious effect on linear child growth, potentially as a result of glutamate's importance in amino acid metabolism, nitrogen balance and barrier function. This observation may also reflect the state of malnutrition in these cohorts of children, as the microbiota turns to host-associated proteins for energy. The weak negative association between Weissella and future HAZ observed in the Bangladesh cohort was not detected in the Malawi children, and needs to be confirmed in other studies.

The impact of Acidaminococcus on growth may also involve its microbial relationships. Network analysis provides a useful framework for identifying important bacteria by their number of relationships (288–290). One study used correlation network centrality measures to identify bacteria that successfully promote the growth conditions of a previously uncultivable microorganism (291). In the Malawi cohort, Acidaminococcus showed a large increase in degree centrality in cases, indicating a potential increase in its influence on microbiota composition. The possibility that rare commensals can promote pathological states based on their relationships with other microbes, despite their low abundance, has been proposed (292) and is in line with the notion of keystone organisms (292–294). Although an increase in Acidaminococcus centrality was not observed in the Bangladesh cases, random sampling error introduced by selecting cases and controls from such a small population (n=25), lacking truly healthy control subjects of normal length, could bias how representative the case and control

exposure histories were in that cohort. Larger epidemiological and experimental investigations are needed to confirm these findings and the mechanisms involved.

Finally in both populations, we observed greater density in case networks that was only statistically significant in the Malawi cohort, and a larger proportion of connections from aerobes to anaerobes in cases. An increase in the average number of connections with worsening nutritional status was also reported in children with SAM using correlation networks (295), and greater connectivity between aerobic and anaerobic bacteria was reported for the microbiota correlation network of children with moderate-to-severe diarrhea compared to non-diarrheal controls (296). Simulation studies suggest that increased density may provide greater resource flow to nodes that are normally of low importance and may reduce the efficiency of resource flow out of the system (297,298).

In construction of our graphical models, we adjusted for potential confounders that were reported (e.g. age and WHZ), but could not control for confounding when comparing case and control network indices. These differences may, therefore, still be confounded by age or by other unreported factors, since controls were older than cases in both populations, and microbiota composition and structure may relate to the timing of complementary food introduction. We cannot dismiss the possibility of spurious associations in our graphical models due to compositional effects (299), residual confounding by diet or other factors, and small sample size. The resulting “noise” limited our ability to detect differences between case and control networks, and we must exercise caution in interpreting pairwise associations as true ecological interactions.

The between-within multivariable regression models, however, control for unreported confounders that are shared between co-twins (e.g. fetal, maternal and environmental); as well as reported confounders that differ between siblings (e.g. diarrhea). Although residual confounding due to unreported factors that may differ between siblings, such as HIV status, is possible (these data were not available from either cohort), the association between

Acidaminococcus and linear growth was reproduced in *both* populations. We also lagged these models so that changes in exposure preceded changes in growth. The temporality adds credibility to our main findings that an increase in Acidaminococcus and glutamate-fermenting microbes are associated with future growth deficits.

Measurement error in quantification of relative abundance is unavoidable in microbiota studies. Since any such error is unlikely to be systematically related to *future* growth deficits *between* siblings, measurement error in these analyses would attenuate true associations with growth, further reducing our power in these small cohorts. Finally, the average child in these populations already suffered from severe growth restriction at study entry, and these data may not elucidate the potential negative effect of microbiota dysbiosis or the protective effect of certain genera in children who are of normal length but still at risk of becoming stunted.

Conclusions

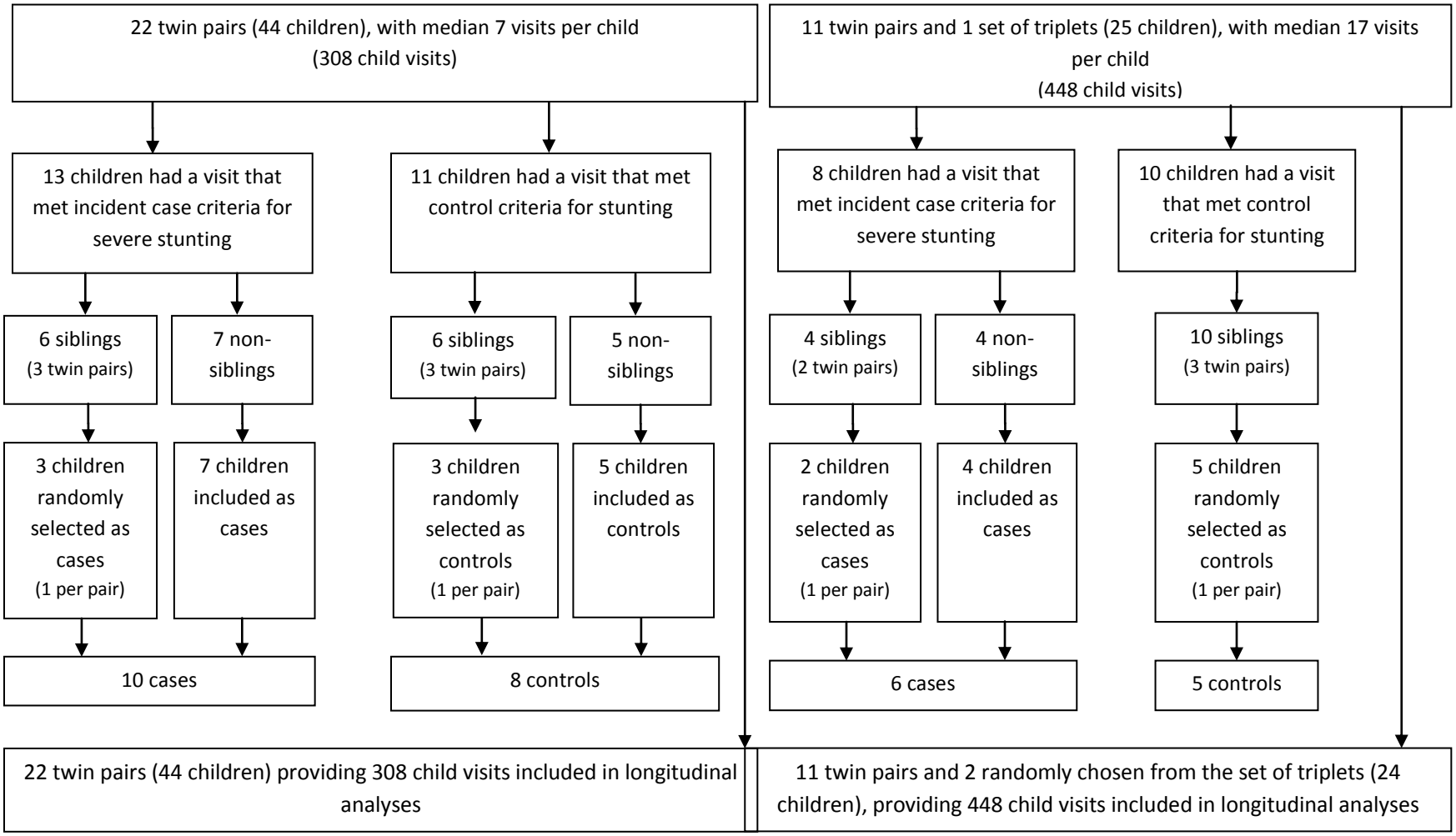
Our study applied a novel use of statistical learning and network methods to identify and interpret changes in graphical models of microbiota covariance patterns. They suggest that reduced microbiota diversity and changes in covariance network density are associated with stunting severity, and that overgrowth of Acidaminococcus, and possibly other glutamate-fermenting microbes, may contribute to future growth deficits in already malnourished children. Our findings demonstrate the potential role that certain types of commensals in the gut may have on linear growth deficits. Larger studies in other settings are needed to confirm these findings, and experimental studies are needed to clarify the mechanisms involved.

12-1 Table 1. Relative Genus Abundance Associations with Future HAZ Estimated Using Multivariable Between-Within Twin Regression Models for Genera with a Significant Difference in Degree Centrality between Cases and Controls.

Genus	Malawi				Bangladesh			
	Abundance Difference*	Coefficient(90%CI)	p-value	Adjusted p-value	Abundance Difference*	Coefficient(90%CI)	p-value	Adjusted p-value
Acidaminococcus	0.40	-0.080(-0.124,-0.037)	<0.01	0.02	0.30	-0.191(-0.253,-0.129)	<0.01	<0.01
Acinetobacter†					0.00	-0.032(-0.159,0.094)	0.68	0.89
Anaerococcus†					0.01	-0.182(-0.915,0.551)	0.68	0.89
Bacteroides	4.51	0.000(-0.001,0.001)	0.67	0.89	0.29	-0.001(-0.002,0.001)	0.63	0.89
Blautia	2.51	-0.001(-0.003,0.002)	0.64	0.89	5.00	0.001(0.000,0.001)	0.07	0.45
Brachyspira	1.03	0.003(-0.002,0.007)	0.32	0.89				
Chlamydiaceae_uncl	0.37	-0.012(-0.054,0.030)	0.65	0.89				
Coprococcus	0.35	-0.006(-0.061,0.049)	0.87	0.92	4.33	-0.003(-0.010,0.003)	0.38	0.89
Geobacillus†					0.01	0.266(-0.154,0.685)	0.30	0.89
Haemophilus	0.76	0.001(-0.009,0.010)	0.92	0.92				
Lactococcus†					0.04	-0.002(-0.007,0.004)	0.59	0.89
Micrococcus†					0.46	-0.107(-2.183,0.169)	0.16	0.94
Neisseriaceae_uncl	0.22	-0.027(-0.103,0.048)	0.56	0.89	0.01	0.001(-0.001,0.004)	0.46	0.64
Proteus†					0.00	-0.002(-0.037,0.033)	0.94	0.94
Sarcina†					5.00	0.000(0.000,0.001)	0.54	0.89

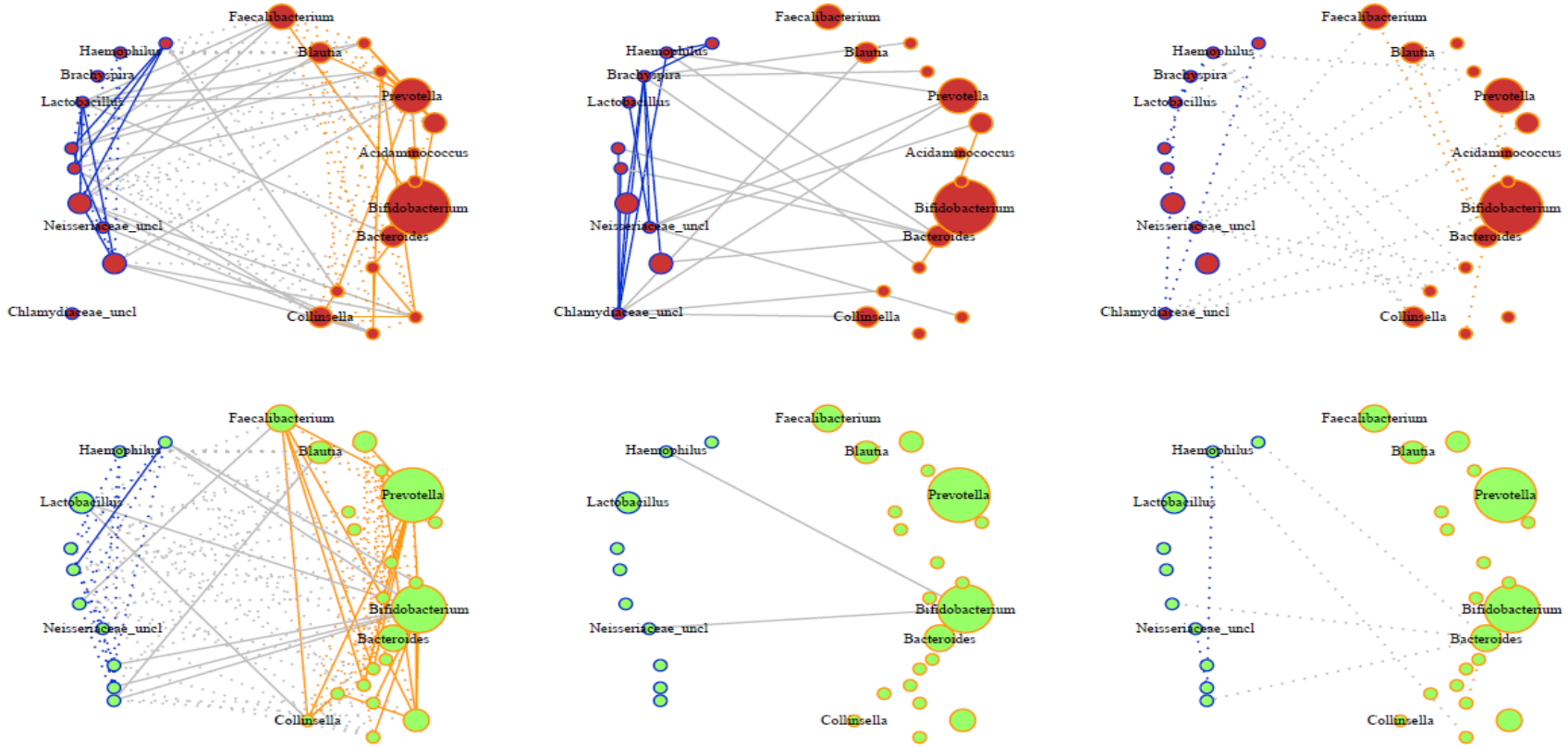
Coefficients are expressed as the average difference in future HAZ per 0.1% difference in abundance between siblings. 90%CI, 90% confidence interval; HAZ, height-for-age z-score. *Median difference in relative abundance between siblings in a twin pair. †Models could not be fit in the Malawi cohort because these genera were only identified in ≤2 samples

12-1 Figure 1. Flow-chart of Case and Control Selection for Network Analysis. (Left) Malawi Twin Cohort. (Right) Bangladesh Twin Cohort.



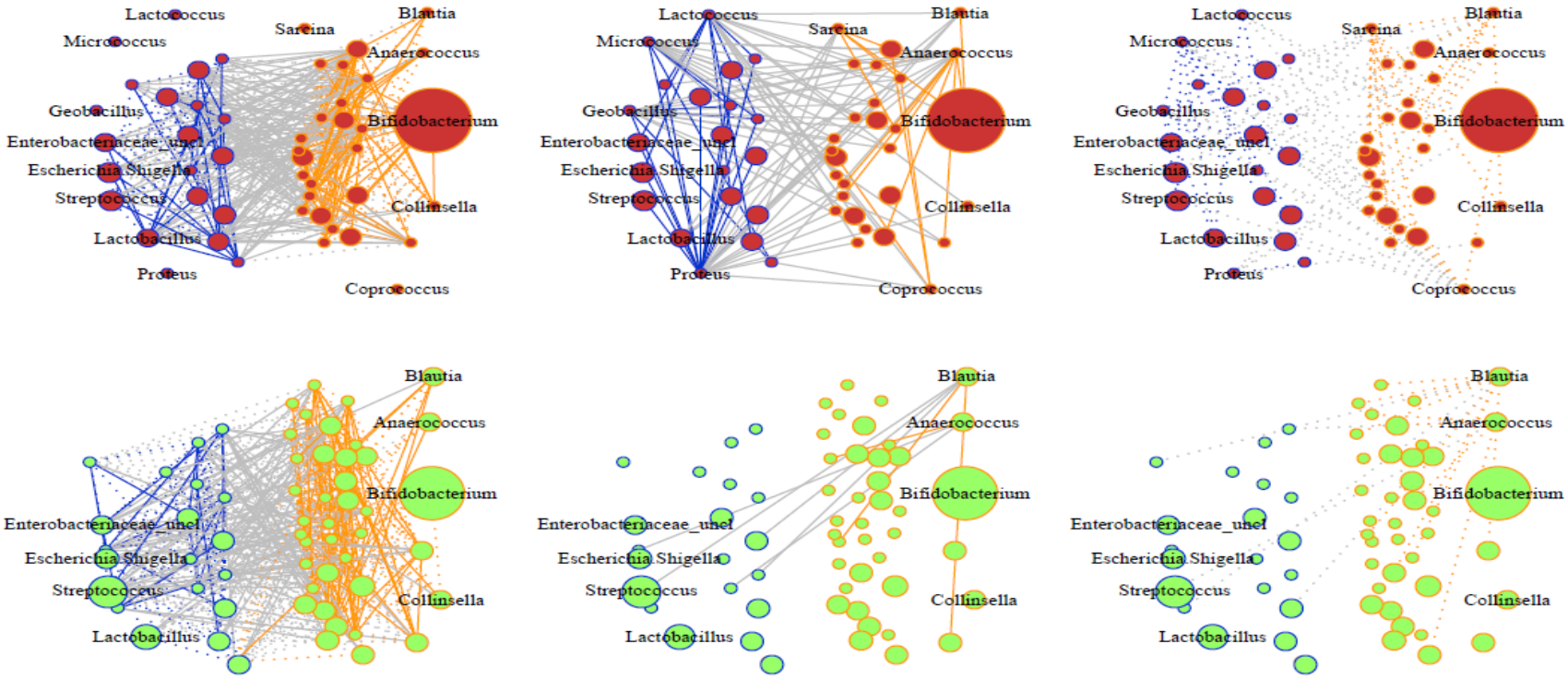
12-2. Figure 2. Graphical Models of Malawi Case and Control Microbiota Networks Constructed Using Glasso.

(Top) Case Networks. (Bottom) Control networks. (Left to Right) Associations found in both groups, cases only, and controls only. Solid and dotted edges indicate positive and negative associations. Blue indicates associations among aerobic and facultative anaerobic genera. Orange indicates associations among anaerobic genera. Gray indicates associations from aerobic/facultative anaerobic to anaerobic genera. Node size is proportional to median abundance.



12-3. Figure 3. Graphical Models of Bangladesh Case and Control Microbiota Networks Constructed Using Glasso.

(Top) Case networks. (Bottom) Control networks. (Left to Right) Associations found in both groups, cases only, and controls only. Solid and dotted edges indicate positive and negative associations. Blue indicates associations among aerobic and facultative anaerobic genera. Orange indicates associations among anaerobic genera. Gray indicates associations from aerobic/facultative anaerobic to anaerobic genera. Node size is proportional to median abundance.



12.3. Appendix 1: Extended Methods

Case-Control Network Analyses

A supplemental approach to diversity indices for investigating the microbiota uses correlation networks as a model of microbe-microbe interactions. Microorganisms in the gut interact in a range of beneficial or antagonistic ecological relationships that arise, for example, through exchange of metabolic products or by-products, competition for nutrients, molecular signaling, or co-aggregation into consortia (300,301). Networks reflecting the pattern of co-occurrence between microbial taxa can be used as a model of bacterial ecological interactions (300,302). Use of such models is based on the premise that non-random patterns in taxon co-occurrence arise through such ecological relationships (303,304). These correlation network models of microbiota interactions have been used by some studies to date to investigate aspects of human microbiota community assembly (302) and its relationship to disease (132,305). We utilize an approach which estimates the covariance structure of abundance data as a graphical model of interactions (as opposed to statistical testing of pairwise correlations), based on the rationale that the covariance structure describes the microbial relationships that give rise to the observed distribution of abundances.

We estimated undirected graphical models from genus abundances, separately for cases and controls using the graphical lasso (glasso) (256). The glasso estimates an inverse covariance matrix from genus abundance data. Each pairwise value in the matrix is adjusted on the abundances of the remaining taxa in the microbiota, and any other covariates included. We included age and WHZ as additional covariates, as well as RUTF treatment in the Malawi data analyses. The algorithm also returns an estimated matrix where weak associations between taxa are shrunk to zero to ensure that insignificant dependences between taxa are excluded. We obtained one matrix for cases and one for controls. Each matrix was transformed into an unweighted, undirected network for subsequent network analyses. In this representation, nodes are genera, and a link between two nodes represents a non-zero association between two genera that is independent of all other taxa identified, age, WHZ, and RUTF treatment; this association is used as a proxy for bacterial interaction. To select the best tuning parameter for

graphical model estimation, we used the Stability Approach for Regularisation Selection method (StARS) (306).

For each case and control network, we calculated graph density, and the normalized degree centrality of each taxon (307). Graph density is expressed as the probability (0 to 1) that two randomly selected nodes are connected, and provides a measure of the potential for information flow (e.g. nutrients, metabolic by-products, and molecular signals) over the network. Degree centrality provides a measure of node importance, based on assumptions regarding how information flows between nodes (308). Assuming that information can flow from a single node to multiple other microbes simultaneously, normalized degree (expressed as the proportion [0 to 1] of other genera that a specific genus is connected to) can be regarded as a measure of a microbe's participation in information flow. A node with a larger number of connections can have greater influence in the network. It thus provides a measure of node importance that is useful for identifying members in a microbial community that can exert a disproportionate impact on its composition and function (307,309,310).

Differences in network indices were assessed for statistical significance by permutation test with 1000 randomizations. Specifically, children were randomly reallocated between the case and control groups 1000 times. For each permutation, one network was estimated per group, as described, and distributions of the difference in network indices between case and control networks were generated for statistical inference. Genera with significant differences in degree centrality or relative abundance between cases and controls were selected for longitudinal analyses.

Longitudinal Analyses to Test a posteriori Hypotheses

We tested our a posteriori hypothesis that glutamate fermentation by microbes is negatively associated with future HAZ using KEGG enzyme abundance data provided for the Malawi cohort at http://gordonlab.wustl.edu/SuppData.html. We fitted between-within regression models, using the relative abundance of genes encoding glutamate dehydrogenase (EC1.4.1.2), α-keto-glutarate reductase (EC1.1.99.2), and methylaspartate mutase (EC5.4.99.1) as exposures. These are critical enzymes in glutamate fermentation pathways used by microbes (286). We fitted a separate model for each gene, with relative abundance as the exposure and

HAZ as the outcome. Each model was adjusted for reported diarrhea, WHZ, and alpha diversity as reported confounders not shared by co-twins. Age in months and length of follow-up since baseline were also included as predictors of the outcome. All covariates were lagged by one visit in order to model their effect on future HAZ, with the exception of length of follow-up and age. Functional gene abundance data were not available for the Bangladesh cohort because only the 16S gene was sequenced.

Reproducibility

Any bacterial genus that was found to have a significant difference in degree centrality or relative abundance between cases and controls in either cohort was investigated in both datasets as a determinant of future linear growth using multivariable between-within regression models. This allowed us to confirm any microbiota associations with future linear growth we identified.

12.4. Appendix 2. Table of Study Participant Characteristics in each Cohort at the Baseline Visit and in Cases versus Controls.

	Malawi				Bangladesh			
	Cohort(n=44)*	**CA(n=10)**	**CO(n=8)**	**p-value†**	**Cohort(n=24)***	**CA(n=6)**	**CO(n=5)**	**p-value†**
Number of Fecal Samples, M[IQR]	7[4,8]				17[13,22]			
Follow-up Time (months), M[IQR]	9.6[4.1,14.5]				14.5[11.9,20.7]			
Age Group, n(%)								
≤6 Months	14(31.82)	3(30.0)	0(0.0)		24(100.0)	6(100.0)	1(20.0)	
6< Months ≤12	10(22.72)	3(30.0)	2(25.0)	0.32	0(0.0)	0(0.0)	4(80.0)	0.04
>12 Months	20(45.45)	4(40.0)	6(75.0)		0(0.0)	0(0.0)	0(0.0)	
Age (months), M[IQR]	10.2[4.6,14.5]	10.8[6.3,18.0]	19.6[12.6,23.8]	0.05	0.26[0.19,0.63]	2.9[2.2,3.7]	11.0[8.1,11.1]	<0.01
Sex, n(%)								
Male	24(54.55)	5 (50.0)	4(50.0)	1.00	7(29.2)	2(33.3)	1(20.0)	1.00
Female	20(45.45)	5 (50.0)	4(50.0)		17(70.8)	4(66.7)	4(80.0)	
WHZ, M[IQR]	-0.46[-0.87,-0.13]	0.23[0.08,0.94]	-0.04[-0.92,0.76]	0.28	-0.57[-1.51,0.35]‡	0.53[0.17,1.23]	-0.64[-1.05,-0.59]	0.05
HAZ, M[IQR]	-2.95[-3.70,-2.18]	-3.08[-3.23,-3.04]	-2.45[-2.60,-2.28]	<0.01	-3.75[-4.54,-2.68]‡	-3.17[-3.37,-3.08]	-2.63[-2.94,-2.29]	<0.01
MUAC, M[IQR]	13.2[12.0,13.8]	13.55[12.65,14.00]	14.70[12.75,15.45]	0.10				
Days of Fever, M[IQR]§	0.0[0.0,2.0]	1.5[1.0,2.7]	0.0[0.0,2.5]	0.16				
Days of Cough, M[IQR]§	0.0[0.0,3.2]	2.5[0.2,4.5]	0.0[0.0,0.75]	0.17				
Days of Vomiting, M[IQR]§	0.0[0.0,0.0]	0.0[0.0,0.0]	0.0[0.0,0.0]	1.00				
Days of Diarrhea, M[IQR]§	0.0[0.0,2.0]	0.0[0.0,0.0]	0.0[0,0.5]	0.98				
Diarrhea, n(%)¶					0(0.0)	0(0.0)	0(0.0)	1.00
Site, n(%)								
Chamba	10(22.73)	2(20.0)	2(25.0)					
Makwhira	4(9.09)	0(0.0)	1(12.5)					
Mayaka	12(27.27)	2(20.0)	3(37.5)	0.72				
Mbiza	16(36.36)	6(60.0)	2(25.0)					
Mitondo	2(4.54)	0(0.0)	0(0.0)					
Antibiotic use, n(%)#					0(0.0)	1(16.7)	2(40.0)	0.85
Diet, n(%)¶								
Breast Milk					23(95.8)	6(100.0)	3(60.0)	0.55
Formula					0(0.0)	6(100.0)	4(80.0)	1.00
Solid Foods					0(0.0)	1(16.7)	3(60.0)	0.39
Simpson Diversity, M[IQR]	0.51[0.25,0.62]	0.50[0.18,0.65]	0.71[0.67,0.77]	0.02	0.52[0.34,0.59]	0.48[0.28, 0.61]	0.70[0.64,0.76]	0.05
Reads per Sample, M[IQR]	76,700[55,200, 103,000]	76,778[62,319, 104,611]	81,897[64,562, 100,075]	0.45	20,192[16,155, 24,632]	19,518[14,711, 25204]	20,592[18,806, 28,481]	0.46

CA, cases (severely stunted); CO, controls (stunted); n, number; M, median; IQR, inter-quartile range, WHZ, weight-for-height z-score; HAZ, height-for-age z-score; MUAC, mid-upper arm circumference.

*Cohort at baseline. †p-value for Cases versus Controls. ‡Measurements at study entry were missing for 22 of 24 children, we report measurements at the second visit. §Prior to the visit. ¶At the visit. #In the 7 days prior to the visit.

12.5. Appendix 3. Genus Relative Abundance and Genus Presence in 308 Malawi and 429 Bangladesh Fecal Samples Collected During Follow-up.

Taxon	Malawi			Bangladesh		
	Relative Abundance	Presence/Absence		Relative Abundance	Presence/Absence	
	M[min,max]	n	% (95%CI)	M[min,max]	n	% (95%CI)
Abiotrophia				0.0[0.0, 0.01]	13	3.03(1.62, 5.13)
Acetobacteraceae_uncl	0.0[0.0, 1.1]	2	0.65(0.08, 2.33)			
Acidaminococcus	0.0[0.0, 2.0]	6	1.95(0.72,4.19)	0.0[0.0, 0.3]	22	5.13(3.24, 7.66)
Acinetobacter				0.0[0.0, 4.6]	101	23.54(19.61, 27.85)
Actinobacillus				0.0[0.0, 2.5]	147	34.27(29.78, 38.97)
Actinomyces				0.01[0.0, 0.9]	252	58.74(53.92, 63.44)
Aerococcus				0.0[0.0, 0.01]	2	0.47(0.06, 1.67)
Aeromonas				0.0[0.0, 0.4]	23	5.36(3.43, 7.94)
Akkermansia	0.0[0.0, 7.1]	14	4.55(2.51, 7.51)	0.0[0.0, 1.4]	15	3.50(1.97, 5.70)
Alcaligenaceae_uncl				0.0[0.0, 0.01]	4	0.93(0.25, 2.37)
Alistipes	0.0[0.0, 1.7]	9	2.92(1.34, 5.47)	0.0[0.0, 0.5]	14	3.26(1.80, 5.41)
Alkaliphilus	0.0[0.0, 0.7]	1	0.32(0.01, 1.80)			
Allisonella				0.0[0.0, 1.4]	73	17.02(13.58, 20.91)
Anaerobiospirillum				0.0[0.0, 3.0]	5	1.17(0.38, 2.70)
Anaerococcus	0.0[0.0, 0.1]	2	0.65(0.08, 2.33)	0.0[0.0, 0.2]	135	31.47(27.10, 36.10)
Anaerofustis				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Anaeroglobus				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Anaerotruncus				0.0[0.00, 0.0]	4	0.93(0.25, 2.37)
Asaccharobacter				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Atopobium	0.0[0.0, 1.5]	3	0.97(0.20, 2.82)	0.0[0.0, 0.2]	112	26.11(22.01, 30.54)
Bacillaceae_uncl				0.0[0.0, 0.7]	58	13.52(10.43, 17.12)
Bacillus				0.0[0.0, 0.0]	7	1.63(0.66, 3.33)
Bacteroides	3.7[0.0,49.8]	237	76.95(71.83,81.53)	0.0 [0.0,21.3]	285	66.43(61.75, 70.89)

Barnesiella				0.0[0.0, 0.1]	4	0.93(0.25, 2.37)
Bifidobacteriaceae_uncl				0.0[0.0, 0.3]	381	88.81(85.44, 91.63)
Bifidobacterium	42.8[0.0,99.6]	287	93.18(89.77,95.73)	46.2[0.0,96.8]	429	100.00(99.14,100.00)
Blautia	0.6[0.0,15.4]	187	60.71(55.02,66.20)	0.0[0.0,55.8]	245	57.11(52.27, 61.85)
Brachyspira	0.0[0.0,17.6]	21	6.82(4.27,10.23)	0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Bulleidia				0.0[0.0, 0.1]	11	2.56(1.29, 4.54)
Butyricicoccus				0.0[0.0, 3.5]	127	29.60(25.32, 34.17)
Butyricimonas				0.0[0.0, 0.1]	2	0.47(0.06, 1.67)
Butyrivibrio	0.0[0.0,15.0]	44	14.29(10.58,18.70)			
Campylobacter	0.0[0.0, 2.3]	8	2.60(1.13, 5.05)	0.0[0.0,10.9]	154	35.90(31.35, 40.64)
Catenibacterium	0.0[0.0, 4.3]	29	9.42(6.40,13.24)	0.0[0.0,12.9]	147	34.27(29.78, 38.97)
Centipeda				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Cetobacterium				0.0[0.0,13.7]	41	9.56(6.95, 12.74)
Chlamydiaceae_uncl	0.0[0.0, 1.9]	37	12.01(8.60,16.18)			
Citrobacter	0.0[0.0, 2.4]	13	4.22(2.27, 7.11)	0.0[0.0, 0.1]	14	3.26(1.80, 5.41)
Clostridiaceae_uncl				0.0[0.0, 2.9]	101	23.54(19.61, 27.85)
Clostridiales_Family_XI_Incertae_Sedis_uncl	0.0[0.0, 0.9]	7	2.27(0.92, 4.63)	0.0[0.0, 0.0]	15	3.50(1.97, 5.70)
Clostridiales_Family_XIII_Incertae_Sedis_uncl				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Clostridium	0.0[0.0,10.6]	7	2.27(0.92, 4.63)	0.0[0.0,57.9]	255	59.44(54.63, 64.12)
Collinsella	1.0[0.0,17.6]	211	68.51(63.00,73.66)	0.5[0.0,15.3]	310	72.26(67.76, 76.45)
Comamonadaceae_uncl				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Comamonas				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Coprobacillus	0.0[0.0, 3.9]	5	1.62(0.53, 3.75)			
Coprococcus	0.0[0.0, 1.1]	21	6.82(4.27,10.23)	0.0[0.0, 4.1]	106	24.71(20.70, 29.07)
Coriobacteriaceae_uncl				0.0[0.0, 10.0]	257	59.91(55.10, 64.58)
Corynebacterium				0.0[0.0, 0.9]	221	51.52(46.67, 56.34)
Dermabacteraceae_uncl				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Dermatophilaceae_uncl				0.0[0.0, 0.0]	4	0.93(0.25, 2.37)
Desulfobulbaceae_uncl	0.0[0.0, 4.8]	112	36.36(30.98,42.01)			
Desulfovibrio	0.0[0.0, 1.7]	13	4.22(2.27, 7.11)	0.0[0.0, 0.0]	4	0.93(0.25, 2.37)

Dialister				0.0[0.0, 9.6]	145	33.80(29.33, 38.49)
Dietzia				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Dolosigranulum				0.0[0.0, 0.1]	53	12.35(9.39, 15.85)
Dorea	0.0[0.0, 4.8]	95	30.84(25.73,36.33)	0.0[0.0,41.5]	267	62.24(57.46, 66.84)
Edwardsiella				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Eggerthella				0.0[0.0, 1.8]	81	18.88(15.29, 22.91)
Enterobacter	0.0[0.0, 3.2]	17	5.52(3.25, 8.69)			
Enterobacteriaceae_uncl				0.4[0.0,69.3]	417	97.20(95.16, 98.55)
Enterococcaceae_uncl				0.0[0.0, 8.6]	260	60.61(55.81, 65.26)
Enterococcus	0.0[0.0, 6.8]	38	12.34(8.88,16.54)	0.0[0.0,33.7]	266	62.00(57.23, 66.62)
Enterorhabdus				0.0[0.0, 0.2]	25	5.83(3.81, 8.48)
Erysipelotrichaceae_uncl				0.0[0.0, 0.8]	70	16.32(12.95, 20.16)
Escherichia	0.0[0.0,44.7]	115	37.34(31.92,43.00)			
Escherichia/Shigella				1.8[0.0,92.5]	423	98.60(96.98, 99.49)
Eubacterium	0.0[0.0,34.4]	77	25.00(20.26,30.23)	0.0[0.0,12.2]	195	45.45(40.67, 50.30)
Exiguobacterium				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Faecalibacterium	3.1[0.0,41.2]	234	75.97(70.80,80.64)	0.0[0.0,24.9]	204	47.55(42.74, 52.40)
Finegoldia				0.0[0.0, 0.4]	107	24.94(20.92, 29.32)
Frankia	0.0[0.0, 0.3]	1	0.32(0.01, 1.80)			
Fusobacteriaceae_uncl				0.0[0.0, 6.1]	66	15.38(12.10, 19.15)
Fusobacterium	0.0[0.0, 1.2]	5	1.62(0.53, 3.75)	0.0[0.0, 0.4]	52	12.12(9.19, 15.59)
Gardnerella	0.0[0.0, 0.01]	3	0.97(0.20, 2.82)	0.0[0.0, 0.0]	4	0.93(0.25, 2.37)
Gemella				0.0[0.0, 0.5]	149	34.73(30.23, 39.45)
Geobacillus				0.0[0.0, 0.3]	18	4.20(2.51, 6.55)
Gordonibacter				0.0[0.0, 0.1]	10	2.33(1.12, 4.24)
Granulicatella				0.0[0.0, 0.1]	94	21.91(18.09, 26.13)
Haemophilus	0.0[0.0, 6.3]	25	8.12(5.32,11.75)			
Hallella				0.0[0.0, 0.0]	4	0.93(0.25, 2.37)
Helicobacter	0.0[0.0, 2.9]	7	2.27(0.92, 4.63)	0.0[0.0, 0.9]	24	5.59(3.62, 8.21)
Holdemania				0.0[0.0, 0.0]	4	0.93(0.25, 2.37)

Howardella				0.0[0.0, 0.0]	21	4.90(3.06, 7.39)
Kingella				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Klebsiella	0.0[0.0, 5.9]	10	3.25(1.57, 5.89)			
Kocuria				0.0[0.0, 1.2]	49	11.42(8.57, 14.82)
Lachnospiraceae_uncl				0.0[0.0, 9.9]	257	59.91(55.10, 64.58)
Lactobacillaceae_uncl				0.0[0.0, 0.7]	230	53.61(48.77, 58.41)
Lactobacillus	0.6[0.0,37.6]	177	57.47(51.73,63.06)	2.6[0.0,77.3]	394	91.84(88.84, 94.25)
Lactococcus	0.0[0.0, 3.3]	1	0.32(0.01, 1.80)	0.0[0.0, 1.0]	98	22.84(18.95, 27.11)
Leptotrichia				0.0[0.0, 1.6]	8	1.86(0.81, 3.64)
Leuconostoc	0.0[0.0, 2.7]	4	1.30(0.35, 3.29)	0.0[0.0, 7.5]	140	32.63(28.21, 37.30)
Leuconostocaceae_uncl				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Macrococcus				0.0[0.0, 0.0]	14	3.26(1.80, 5.41)
Megamonas	0.0[0.0,32.3]	41	13.31(9.72,17.62)	0.0[0.0, 8.6]	149	34.73(30.23, 39.45)
Megasphaera	0.0[0.0, 2.6]	11	3.57(1.80, 6.30)	0.0[0.0,19.8]	188	43.82(39.07, 48.66)
Methanobrevibacter	0.0[0.0, 0.5]	1	0.32(0.01, 1.80)	0.0[0.0, 0.1]	4	0.93(0.25, 2.37)
Methanosphaera				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Micrococcaceae_uncl				0.0[0.0, 0.0]	25	5.83(3.81, 8.48)
Micrococcus	0.0[0.0, 0.01]	1	0.32(0.01, 1.80)	0.0[0.0, 0.0]	9	2.10(0.96, 3.94)
Mitsuokella	0.0[0.0,10.1]	37	12.01(8.60,16.18)	0.0[0.0, 2.2]	21	4.90(3.06, 7.39)
Mobiluncus				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Mogibacterium				0.0[0.0, 0.1]	19	4.43(2.69, 6.83)
Moraxella				0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Moraxellaceae_uncl				0.0[0.0, 0.0]	5	1.17(0.38, 2.70)
Mycobacterium	0.0[0.0, 2.1]	86	27.92(22.98,33.29)	0.0[0.0, 0.0]	1	0.23(0.01, 1.29)
Neisseria	0.0[0.0, 1.1]	3	0.97(0.20, 2.82)	0.0[0.0, 0.3]	59	13.75(10.64, 17.38)
Neisseriaceae_uncl	0.0[0.0, 1.2]	5	1.62(0.53, 3.75)	0.0[0.0, 0.0]	6	1.40(0.51, 3.02)
Nocardiopsis				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Odoribacter	0.0[0.0, 1.5]	5	1.62(0.53, 3.75)	0.0[0.0, 0.0]	6	1.40(0.51, 3.02)
Olsenella	0.0[0.0, 0.05]	2	0.65(0.08, 2.33)	0.0[0.0,20.7]	242	56.41(51.57, 61.16)
Oribacterium				0.0[0.0, 0.0]	6	1.40(0.51, 3.02)

Oscillibacter				0.0[0.0, 3.1]	39	9.09(6.54, 12.22)
Oxalobacteraceae_uncl				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Parabacteroides	0.0[0.0, 3.3]	15	4.87(2.75, 7.91)	0.0[0.0, 0.2]	46	10.72(7.96, 14.04)
Paracoccus				0.0[0.0, 0.0]	6	1.40(0.51, 3.02)
Paraprevotella				0.0[0.0, 0.0]	3	0.70(0.14, 2.03)
Parasutterella				0.0[0.0, 0.5]	8	1.86(0.81, 3.64)
Parvimonas				0.0[0.0, 0.1]	45	10.49(7.75, 13.78)
Pasteurellaceae_uncl				0.0[0.0, 0.2]	83	19.35(15.72, 23.41)
Pediococcus				0.0[0.0, 1.1]	8	1.86(0.81, 3.64)
Peptococcus				0.0[0.0, 0.1]	18	4.20(2.51, 6.55)
Peptoniphilus	0.0[0.0, 0.6]	4	1.30(0.35, 3.29)	0.0[0.0, 0.1]	118	27.51(23.33, 31.99)
Peptostreptococcaceae_uncl				0.0[0.0, 0.0]	4	0.93(0.25, 2.37)
Peptostreptococcus				0.0[0.0, 1.0]	74	17.25(13.79, 21.16)
Phascolarctobacterium	0.0[0.0, 6.7]	20	6.49(4.01, 9.85)	0.0[0.0, 0.0]	5	1.17(0.38, 2.70)
Porphyromonadaceae_uncl				0.0[0.0, 0.1]	8	1.86(0.81, 3.64)
Porphyromonas				0.0[0.0, 0.0]	31	7.23(4.96, 10.10)
Prevotella	22.7[0.0,80.5]	261	84.74(80.23,88.57)	0.0[0.0,36.6]	316	73.66(69.22, 77.77)
Prevotellaceae_uncl				0.0 [0.0, 5.0]	114	26.57(22.45, 31.02)
Proteus				0.0[0.0, 1.3]	19	4.43(2.69, 6.83)
Providencia	0.0[0.00, 0.7]	1	0.32(0.01, 1.80)	0.0[0.0,57.0]	10	2.33(1.12, 4.24)
Pseudobutyrivibrio				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Pseudomonas	0.0[0.0, 0.3]	2	0.65(0.08, 2.33)	0.0[0.0, 0.0]	4	0.93(0.25, 2.37)
Roseburia	0.0[0.0,16.6]	74	24.03(19.36,29.20)	0.0 [0.0,23.0]	139	32.40(27.99, 37.06)
Rothia	0.0[0.00, 0.7]	4	1.30(0.35, 3.29)	0.0[0.0, 3.0]	331	77.16(72.89, 81.05)
Ruminococcaceae_uncl				0.0[0.0, 5.6]	122	28.44(24.21, 32.96)
Ruminococcus	0.0[0.00,30.7]	123	39.94(34.42,45.64)	0.0[0.0, 4.2]	54	12.59(9.60, 16.10)
Sarcina				0.0[0.0,23.7]	59	13.75(10.64, 17.38)
Shigella	0.0[0.0, 9.3]	18	5.84(3.50, 9.08)			
Slackia	0.0[0.0, 1.9]	18	5.84(3.50, 9.08)	0.0[0.0, 0.3]	78	18.18(14.65, 22.16)
Solobacterium				0.0[0.0, 0.0]	4	0.93(0.25, 2.37)

Sphingobacteriaceae_uncl	0.0[0.0, 5.8]	73	23.70(19.06,28.85)			
Sporobacter				0.0[0.0, 0.2]	11	2.56(1.29, 4.54)
Staphylococcaceae_uncl				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Staphylococcus	0.0[0.0, 6.8]	1	0.32(0.01, 1.80)	0.0[0.0,19.0]	202	47.09(42.28, 51.93)
Stenotrophomonas				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Streptobacillus				0.0[0.0, 0.0]	2	0.47(0.06, 1.67)
Streptococcaceae_uncl				0.0[0.0, 7.07]	201	46.85(42.05, 51.70)
Streptococcus	0.0[0.0,86.3]	149	48.38(42.67,54.11)	4.8[0.0,94.7]	429	100.00(99.14,100.00)
Streptophyta_uncl				0.0[0.0, 0.0]	28	6.53(4.38, 9.30)
Subdoligranulum	0.0[0.0, 0.5]	6	1.95(0.72, 4.19)	0.0[0.0,17.8]	130	30.30(25.99, 34.89)
Succinivibrio				0.0[0.0, 0.7]	24	5.59(3.62, 8.21)
Sutterella	0.0[0.0, 1.6]	26	8.44(5.59,12.12)	0.0[0.0, 2.1]	100	23.31(19.39, 27.60)
Tepidibacter				0.0[0.0, 0.1]	3	0.70(0.14, 2.03)
Treponema				0.0[0.0, 0.3]	3	0.70(0.14, 2.03)
Turicibacter				0.0[0.0, 0.3]	19	4.43(2.69, 6.83)
Unclassified	0.0[0.0, 0.8]	13	4.22(2.27, 7.11)	1.5[0.2,60.9]	429	100.00(99.14,100.00)
Ureaplasma				0.0[0.0, 0.0]	26	6.06(4.00, 8.75)
Varibaculum				0.0[0.0, 0.2]	39	9.09(6.54, 12.22)
Veillonella	0.0[0.0,28.8]	138	44.81(39.16,50.55)	0.0[0.0, 9.3]	322	75.06(70.68, 79.08)
Veillonellaceae_uncl				0.0[0.0,15.7]	156	36.36(31.80, 41.11)
Victivallis	0.0[0.00, 0.3]	1	0.32(0.01, 1.80)	0.0[0.0, 0.0]	0	0.00(0.00, 0.86)
Weissella				0.0[0.0,12.0]	201	46.85(42.05, 51.70)

M, Median

12.6. Appendix 4. Relative Abundance and Normalized Degree Centrality of Genera Identified in Severely Stunted Cases and Stunted Controls selected from the Malawi Cohort.

Taxon	Median Abundance [Min,Max]			Normalized Degree Centrality		
	Cases (Severely Stunted); n=10	Controls (Stunted); n=8	p-value	Cases (Severely Stunted); n=10	Controls (Stunted); n=8	p-value
Acidaminococcus	0.00[0.00, 0.92]	0.00[0.00, 0.00]	1.00	0.56	0.00	0.06
Anaerococcus	0.00[0.00, 0.00]	0.00[0.00, 0.15]	1.00	0.00	0.29	0.69
Bacteroides	1.90[0.00, 8.62]	7.36[1.86,14.52]	0.01	0.60	0.23	0.03
Bifidobacterium	44.94[0.00,94.89]	34.97[0.00,40.06]	0.10	0.32	0.45	0.46
Blautia	0.55[0.00, 8.93]	2.43[0.52, 5.90]	0.03	0.48	0.23	0.27
Brachyspira	0.00[0.00, 0.71]	0.00[0.00, 0.00]	1.00	0.56	0.00	0.09
Butyrivibrio	0.00[0.00, 1.43]	0.00[0.00, 1.08]	1.00	0.48	0.26	0.45
Chlamydiaceae_uncl	0.00[0.00, 0.36]	0.00[0.00, 0.00]	1.00	0.60	0.00	0.05
Collinsella	0.58[0.00, 6.02]	0.00[0.00, 4.18]	0.34	0.56	0.29	0.22
Coprobacillus	0.00[0.00, 0.00]	0.00[0.00, 0.24]	1.00	0.00	0.29	0.69
Coprococcus	0.00[0.00, 0.00]	0.00[0.00, 0.10]	1.00	0.00	0.10	0.94
Desulfobulbaceae_uncl	0.00[0.00, 1.09]	0.00[0.00, 1.70]	0.65	0.60	0.23	0.14
Dorea	0.00[0.00, 0.11]	0.00[0.00, 1.19]	0.98	0.68	0.35	0.19
Enterococcus	0.00[0.00, 0.19]	0.00[0.00, 0.60]	1.00	0.56	0.29	0.25
Escherichia	0.11[0.00, 5.09]	0.00[0.00, 1.23]	0.28	0.64	0.29	0.16
Eubacterium	0.00[0.00, 1.98]	2.37[0.00,12.93]	<0.01	0.48	0.29	0.48
Faecalibacterium	5.89[0.00,15.43]	8.00[1.51,14.37]	0.28	0.48	0.26	0.29
Haemophilus	0.00[0.00, 0.82]	0.00[0.00, 0.66]	1.00	0.64	0.23	0.07
Lactobacillus	0.00[0.00, 6.71]	1.25[0.00, 4.49]	0.19	0.60	0.26	0.15
Leuconostoc	0.00[0.00, 0.00]	0.00[0.00, 2.72]	1.00	0.00	0.23	0.70
Megamonas	0.00[0.00, 6.43]	0.00[0.00,14.13]	0.97	0.56	0.29	0.27
Mitsuokella	0.00[0.00, 0.00]	0.00[0.00, 0.52]	1.00	0.00	0.19	0.68
Mycobacterium	0.00[0.00, 0.32]	0.00[0.00, 0.71]	0.90	0.44	0.13	0.21

Neisseria	0.00[0.00, 0.00]	0.00[0.00, 0.64]	1.00	0.00	0.23	0.94
Neisseriaceae_uncl.	0.00[0.00, 0.10]	0.00[0.00, 1.21]	1.00	0.68	0.23	0.08
Parabacteroides	0.00[0.00, 0.00]	0.00[0.00, 0.19]	1.00	0.00	0.03	0.99
Peptoniphilus	0.00[0.00, 0.00]	0.00[0.00, 0.54]	1.00	0.00	0.13	0.72
Phascolarctobacterium	0.00[0.00, 0.00]	0.00[0.00, 0.78]	1.00	0.00	0.32	0.26
Prevotella	18.12[0.00,71.31]	42.86[11.68,49.90]	0.06	0.52	0.35	0.38
Roseburia	0.00[0.00, 5.15]	0.00[0.00, 1.58]	0.90	0.48	0.13	0.16
Ruminococcus	0.00[0.00, 2.39]	0.04[0.00, 7.48]	0.27	0.52	0.26	0.14
Sphingobacteriaceae_uncl	0.00[0.00, 2.06]	0.00[0.00, 1.16]	0.81	0.52	0.32	0.45
Streptococcus	0.17[0.00,11.09]	0.00[0.00,12.99]	0.34	0.52	0.26	0.22
Veillonella	0.05[0.00,12.63]	0.00[0.00, 3.65]	0.46	0.44	0.13	0.27

12.7. Appendix 5. Relative Abundance and Normalized Degree Centrality of Genera Identified in Severely Stunted Cases and Stunted Controls selected from the Bangladesh Cohort.

Taxon	Median Abundance [Min,Max]			Normalized Degree Centrality		
	Cases (Severely Stunted); n=6	Controls (Stunted); n=5	p-value	Cases (Severely Stunted); n=6	Controls (Stunted); n=5	p-value
Acidaminococcus	0.00[0.00,0.00]	0.00[0.00,0.31]	1.00	0.00	0.39	0.49
Acinetobacter	0.00[0.00,0.01]	0.00[0.00,0.00]	1.00	0.53	0.00	0.03
Actinobacillus	0.00[0.00,0.00]	0.00[0.00,0.01]	1.00	0.00	0.38	0.52
Actinomyces	0.00[0.00,0.10]	0.00[0.00,0.01]	0.36	0.49	0.31	0.51
Allisonella	0.00[0.00,0.00]	0.00[0.00,0.005]	1.00	0.00	0.38	0.52
Anaerococcus	0.00[0.00,0.01]	0.00[0.00,0.03]	0.04	0.71	0.21	0.09
Atopobium	0.00[0.00,0.03]	0.00[0.00,0.03]	0.94	0.78	0.33	0.22
Bacillaceae_uncl	0.00[0.00,0.00]	0.00[0.00,0.07]	1.00	0.00	0.16	0.69
Bacillus	0.00[0.00,0.00]	0.00[0.00,0.005]	1.00	0.00	0.38	0.52
Bacteroides	0.53[0.00,21.33]	0.00[0.00,2.73]	0.28	0.43	0.30	0.60
Bifidobacteriaceae_uncl	0.01[0.00,0.10]	0.07[0.02,0.09]	0.07	0.57	0.23	0.22
Bifidobacterium	67.55[0.06,93.20]	48.89[20.88,93.68]	0.18	0.43	0.21	0.44
Blautia	0.00[0.00,0.01]	0.19[0.00,5.91]	<0.01	0.71	0.21	0.08
Campylobacter	0.00[0.00,0.00]	0.00[0.00,0.33]	1.00	0.00	0.26	0.43
Catenibacterium	0.00[0.00,0.002]	0.00[0.00,0.005]	1.00	0.59	0.38	0.67
Clostridiaceae_uncl	0.00[0.00,0.00]	0.00[0.00,0.01]	1.00	0.00	0.41	0.23
Clostridium	0.01[0.00,52.05]	0.00[0.00,0.76]	0.35	0.59	0.33	0.27
Collinsella	0.00[0.00,8.86]	0.00[0.00,0.74]	0.12	0.55	0.25	0.37
Coprococcus	0.00[0.00,0.01]	0.00[0.00,0.00]	1.00	0.53	0.00	0.03
Coriobacteriaceae_uncl	0.00[0.00,0.01]	0.29[0.00,0.94]	<0.01	0.55	0.33	0.35
Corynebacterium	0.00[0.00,0.03]	0.00[0.00,0.07]	0.38	0.27	0.30	0.91
Dialister	0.00[0.00,0.00]	0.00[0.00,0.16]	<0.01	0.00	0.25	0.45
Dolosigranulum	0.00[0.00,0.002]	0.00[0.00,0.02]	0.94	0.59	0.38	0.52

Dorea	0.00[0.00,0.84]	0.75[0.00,12.88]	0.05	0.55	0.30	0.27
Eggerthella	0.00[0.00,0.22]	0.00[0.00,0.01]	0.95	0.49	0.39	0.77
Enterobacteriaceae_uncl	0.36[0.12,10.08]	0.38[0.06,1.74]	0.42	0.43	0.34	0.70
Enterococcaceae_uncl	0.00[0.00,2.42]	0.14[0.00,0.48]	0.08	0.43	0.36	0.77
Enterococcus	0.78[0.00,5.80]	0.41[0.01,5.83]	0.34	0.20	0.18	0.94
Erysipelotrichaceae_uncl	0.00[0.00,0.01]	0.00[0.00,0.02]	1.00	0.55	0.33	0.72
Escherichia/Shigella	3.17[10.16,18.55]	2.12[1.15,4.71]	0.26	0.55	0.34	0.50
Eubacterium	0.00[0.00,0.00]	0.00[0.00,0.17]	1.00	0.00	0.30	0.39
Faecalibacterium	0.00[0.00,0.002]	0.01[0.00,0.57]	<0.01	0.59	0.36	0.50
Finegoldia	0.00[0.00,0.01]	0.00[0.00,0.003]	0.94	0.45	0.33	0.84
Fusobacteriaceae_uncl	0.00[0.00,0.02]	0.00[0.00,0.05]	0.95	0.55	0.25	0.32
Gemella	0.00[0.00,0.01]	0.005[0.00,0.06]	0.18	0.67	0.33	0.22
Geobacillus	0.00[0.00,0.01]	0.00[0.00,0.00]	1.00	0.59	0.00	0.09
Granulicatella	0.00[0.00,0.00]	0.00[0.00,0.01]	0.95	0.55	0.26	0.31
Helicobacter	0.00[0.00,0.00]	0.00[0.00,0.24]	1.00	0.00	0.38	0.52
Lachnospiraceae_uncl	0.00[0.00,0.03]	0.10[0.05,0.73]	<0.01	0.55	0.26	0.13
Lactobacillaceae_uncl	0.00[0.00,0.02]	0.05[0.00,0.20]	<0.01	0.55	0.39	0.55
Lactobacillus	0.07[0.00,1.43]	8.73[0.01,31.33]	<0.01	0.65	0.38	0.23
Lactococcus	0.00[0.00,0.02]	0.00[0.00,0.00]	1.00	0.65	0.00	0.02
Leuconostoc	0.00[0.00,0.03]	0.00[0.00,0.20]	0.94	0.55	0.39	0.76
Megamonas	0.00[0.00,0.04]	0.01[0.00,1.66]	0.05	0.71	0.39	0.29
Megasphaera	0.00[0.00,0.20]	0.01[0.005,12.01]	0.11	0.71	0.30	0.11
Micrococcus	0.00[0.00,0.01]	0.00[0.00,0.00]	1.00	0.51	0.00	0.05
Mitsuokella	0.00[0.00,0.00]	0.00[0.00,0.44]	1.00	0.00	0.38	0.52
Olsenella	0.00[0.00,0.07]	0.82[0.00,1.50]	<0.01	0.55	0.46	0.74
Peptoniphilus	0.00[0.00,0.00]	0.00[0.00,0.03]	1.00	0.00	0.33	0.45
Peptostreptococcus	0.00[0.00,0.00]	0.00[0.00,0.04]	1.00	0.00	0.33	0.45
Porphyromonas	0.00[0.00,0.00]	0.00[0.00,0.004]	1.00	0.00	0.39	0.49
Prevotella	0.00[0.00,4.17]	0.00[0.00,20.56]	0.47	0.51	0.36	0.58
Proteus	0.00[0.00,0.01]	0.00[0.00,0.00]	1.00	0.59	0.00	0.09

Providencia	0.00[0.00,0.00]	0.00[0.00,0.02]	1.00	0.00	0.13	0.62
Roseburia	0.00[0.00,0.00]	0.00[0.00,0.26]	1.00	0.59	0.38	0.67
Rothia	0.04[0.01,0.76]	0.03[0.00,0.21]	0.35	0.37	0.13	0.32
Ruminococcaceae_uncl	0.00[0.00,0.004]	0.00[0.00,0.003]	1.00	0.55	0.33	0.63
Ruminococcus	0.00[0.00,0.00]	0.00[0.00,0.004]	1.00	0.00	0.39	0.49
Sarcina	0.00[0.00,0.002]	0.00[0.00,0.00]	1.00	0.59	0.00	0.09
Staphylococcus	0.01[0.00,0.24]	0.00[0.00,0.03]	0.46	0.63	0.33	0.50
Streptococcaceae_uncl	0.00[0.00,0.06]	0.02[0.00,0.07]	0.30	0.43	0.28	0.52
Streptococcus	3.13[0.602,29.66]	18.07[2.45,29.42]	0.18	0.45	0.34	0.77
Sutterella	0.00[0.00,0.04]	0.00[0.00,0.005]	0.93	0.59	0.41	0.57
unclassified	0.70[0.32,10.55]	3.08[0.78,8.06]	0.12	0.41	0.41	0.99
Ureaplasma	0.00[0.00,0.00]	0.00[0.00,0.004]	1.00	0.00	0.39	0.49
Veillonella	0.10[0.00,0.80]	0.00[0.00,0.62]	0.35	0.71	0.43	0.39
Veillonellaceae_uncl	0.00[0.00,0.00]	0.00[0.00,0.27]	<0.01	0.00	0.30	0.22
Weissella	0.00[0.00,0.00]	0.05[0.00,0.46]	<0.01	0.00	0.26	0.37

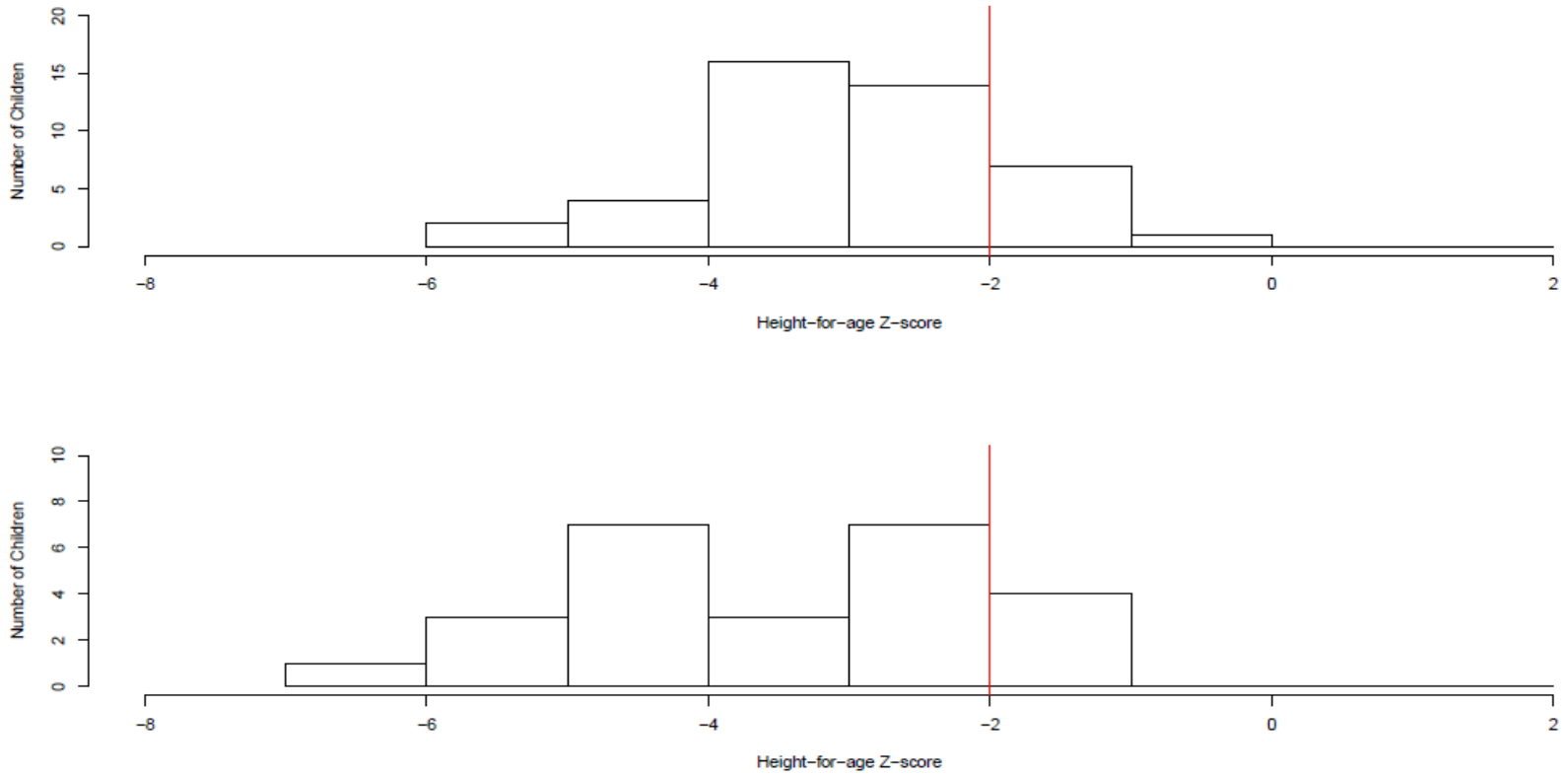
12.8. Appendix 6. Relative Genus Abundance Associations with Future HAZ Estimated Using Multivariable Between-Within Twin Regression Models for Genera with a Significant Difference in Relative Abundance between Cases and Controls.

Genus	Malawi				Bangladesh			
	Abundance Difference*	Coefficient(90%CI)	p-value	Adjusted p-value	Abundance Difference*	Coefficient(90%CI)	p-value	Adjusted p-value
Bifidobacteriaceae_uncl†					0.35	0.033(-0.078,0.143)	0.63	0.92
Coriobacteriaceae_uncl†					2.06	-0.001(-0.005,0.004)	0.82	0.92
Dialister†					3.69	-0.001(-0.004,0.003)	0.73	0.92
Dorea	0.53	-0.001(-0.010,0.008)	0.91	0.91	3.54	0.002(0.000,0.004)	0.10	0.58
Enterococcaceae_uncl†					0.01	0.002(-0.011,0.014)	0.81	0.92
Eubacterium	5.06	0.000(-0.001,0.000)	0.48	0.78	4.10	0.002(-0.005,0.008)	0.68	0.92
Faecalibacterium	5.47	0.000(-0.001,0.002)	0.68	0.78	4.47	0.000(-0.002,0.002)	0.97	1.00
Lachnospiraceae_uncl†					4.33	0.000(-0.003,0.003)	1.00	1.00
Lactobacillaceae_uncl†					0.46	-0.007(-0.043,0.030)	0.77	0.92
Lactobacillus	7.25	0.000(-0.001,0.001)	0.49	0.78	0.39	0.000(-0.001,0.001)	0.71	0.92
Megamonas	1.96	0.002(-0.002,0.005)	0.41	0.78	2.06	-0.424(-9.736,8.887)	0.94	0.92
Olsenella†					0.50	0.000(0.000,0.001)	0.37	0.92
Prevotella	12.48	0.000(-0.001,0.000)	0.32	0.78	1.94	0.000(0.000,0.001)	0.37	0.92
Veillonellaceae_uncl†					3.21	-0.001(-0.003,0.000)	0.18	0.83
Weissella†					0.46	-0.003(-0.004,-0.002)	<0.01	<0.01

Coefficients are expressed as the average difference in future HAZ per 0.1% difference in abundance between siblings. 90%CI: 90% confidence interval, HAZ: height-for-age z-score. *Median difference in relative abundance between siblings in a twin pair. †Models could not be fit in the Malawi cohort because these genera were only identified in ≤2 samples.

12.9. Appendix 7. Height-for-age Z-score Distributions in Children at Study Entry.

(Top) Malawi Cohort, n=44. (Bottom) Bangladesh Cohort, n=24. Red vertical lines indicate the World Health Organization cut-off for Stunting.



13. CONCLUSIONS

Malnutrition is responsible for millions of deaths in children under five years old in LMICs. Some LMICs have made considerable progress in reducing the prevalence of child stunting. However, large socioeconomic disparities persist, and the absolute number of stunted children in some countries is expected to increase with current trends in population growth. Global targets to reduce stunting by 40% by 2025 have been set by the World Health Assembly, but estimates suggest that several countries may fall short of meeting this goal (4).

Growth deficits in child height or length rapidly accumulate in the first thousand days of life. This period is considered a critical window for intervention to reduce child stunting and associated long-term health outcomes. However, estimates of the impact of the interventions most supported by experimental evidence to reduce stunting are modest. These interventions predominantly include zinc supplementation, and complementary feeding coupled with maternal education, both targeted at infants older than six months of age. Evidence for the effectiveness of other interventions has so far been inconclusive. There is a clear need to better understand the etiology of child stunting to inform new and more effective strategies for management and prevention.

Recent publications have drawn attention to the timing of growth faltering and recovery, and renewed discussions around the most appropriate windows of opportunity for intervention (168,311–314). These discussions have reaffirmed that the first thousand days, from conception through 24 months of infant age, is the most critical period. However, the most appropriate timing of interventions within this thousand day window has not received attention. RCTs of zinc supplementation suggest that the effect of zinc on linear growth in children 6-12 months of age is very limited (315), although zinc supplementation in children older than 12 months is deemed to be beneficial (316). A recent RCT also suggested that deworming in pre-school children may be more effective for improving linear growth if implemented at 12 months of age rather than at 18 months (103). Meta-analyses of observational international adoption studies

found that children adopted before 12 months of age had greater linear catch-up growth over an average period of eight years with their adoptive families, compared with children adopted at an older age (317). The timing of interventions within the critical window is likely to be important for optimal growth benefits, and the optimal infant age at which to implement various interventions should be informed by a better understanding of the patterns of linear faltering.

This thesis makes an important contribution to the fundamental question of when growth faltering begins. I identified five distinct growth trajectories among infants followed up from birth through their second birthday. These trajectories were all characterised by worsening linear growth restriction, but varied in the timing and steepness of growth declines. Two groups of infants (D and E) showed very early and rapid growth faltering. Approximately one quarter to one third of infants in either group were stunted by six months of age. Another two groups of infants (B and C) showed slower declines and had a relatively low prevalence of stunting until the end of follow-up, at which time the prevalence of stunting became similar to that observed in Group D. Infants in Group A showed the least severe growth faltering overall. Declines in linear growth in Group A were preceded by a period of apparent healthy growth until age six months, and the prevalence of stunting did not reach the levels observed in the other groups.

Differences in the timing of growth faltering and trends in stunting prevalence between groups suggest different interventional needs. Complementary feeding interventions that target infants between 6-24 months of age, and zinc supplementation at age 12 months may be less effective in infants who accumulate growth deficits earlier. Infants in Groups D and E combined represented one in four children in the ZVITAMBO cohort, which is a substantial number of infants for whom complementary feeding and zinc supplementation after age six months may be too late. The determinants of infant growth trajectory membership which this thesis identified suggest that maternal education, maternal nutrition and intrauterine growth may be most important to longitudinal linear growth in infants. In RCTs, dietary supplementation to pregnant women improves fetal growth, as measured by birthweight and risk of having an SGA

infant (69–72), reduces risk of stunting at age 12 months, and improves attained height in childhood (73,74). Coffey (2015) estimated that limited weight gain during pregnancy as well as poor prepregnancy maternal weight are important contributors to the higher rates of stunting in Indian children, compared to children in sub-Saharan Africa, despite greater poverty in sub-Saharan African countries (67). An RCT conducted in Guatemala found that nutritional supplementation to girls 7-15 years of age produced significant improvements in the birthweight, height, weight and z-scores of their 0-12 year old offspring, 29-38 years after the supplement was given (318). The offspring of girls exposed to the supplement had a 116 g (95%CI: 17 g, 215 g) higher birthweight, were 1.3 cm (0.4 cm, 2.2 cm) taller, had a 0.6 cm (0.4 cm, 0.9 cm) greater head circumference, had a 0.26 (0.09, 0.43) greater HAZ, and had a 0.20 (0.02, 0.39) greater WAZ. Nutritional interventions to mothers should be implemented during pregnancy, but should also target pre-conception in order to more positively impact infant growth and interrupt the intergenerational cycle of stunting.

In addition to dietary supplementation to women, and complementary feeding starting at six months of infant age, improved access to clean water, sanitation and hygiene is also likely to contribute to healthy infant growth. Analyses of Demographic and Health Survey data from 65 countries (319), India (319,320), Bangladesh (321) and sub-Saharan Africa (319) show an inverse association between the prevalence of open defecation and HAZ. Multi-country cohort data from a large study of enteric infections, EED and malnutrition in LMICs show that ≥80% of infants in these cohorts have been infected with at least one enteric pathogen by age six months, and all have been infected with at least one pathogen by 12 months (190). The high carriage of enteric pathogens is often asymptomatic, and may not produce diarrheal episodes until multiple pathogens are present (191). It has been suggested that persistent exposure to enteric pathogens resulting from poor sanitation, even in the absence of diarrheal illness, leads to EED, a sub-clinical condition characterized by histological changes to the gut wall of the small intestine, reduced nutrient absorption, increased gut wall permeability, and systemic inflammation (322–324). Biological markers of EED are associated with poor linear growth (86–89,95). A recent meta-analysis of randomized WASH interventions in children younger than five

years old found a 0.08 standard deviation increase in HAZ (95%CI: 0.00 to 0.16) (22). Stratifying by ages <24 and ≥24 months showed similar gains in HAZ in the intervention arms. However, the effect in children <24 months was not statistically significant, perhaps due to a 54% smaller sample size in this age group. Benefits of improved WASH to the developing fetus are also plausible, since WASH interventions reduce persistent exposure to enteric pathogens for the pregnant mother and infant alike. Large, sufficiently powered RCTs are currently underway to determine the independent and combined impact of WASH interventions implemented during pregnancy, and nutritional supplementation to infants given from age 6-24 months, on infant growth (187–189).

Another contribution of this thesis is the finding that, on average, antibiotics also provide a benefit to growth in children. Antibiotics may improve growth by resolving sub-clinical and clinical infections, which can impair growth via nutrient malabsorption, increased nutrient loss during episodes of diarrhea, gut inflammation, impaired intestinal barrier function, diversion of nutrients away from growth to support immune activation, and loss of appetite (78–80). Growth benefits associated with antibiotic use may also result from alteration of the intestinal microbiota (18,19,147). This explanation has gained traction (164,325), given the results of recent experimental studies that showed a causal effect of the microbiota on weight gain in mice (14,15). One way in which antibiotics impact the microbiota is through the loss of antibiotic-susceptible microbes, which creates an ecological niche that can be occupied by growth of those microbes that have survived antibiotic treatment. However, available niches can be occupied by either beneficial (326) or pathogenic bacteria (327). Infections with enteric pathogens are also temporally associated with shifts in gut microbiota composition in adults (328). In LMICs, where enteric infections are common-place and occur very early in infancy, antibiotic use may help to preserve a relatively pathogen-free gut microbiota, minimize pathogen associated changes, and preserve commensal bacterial functions such as nutrient absorption and energy harvesting.

However, at this stage antibiotics are only recommended for hospital-based treatment of SAM, not for routine community-based treatment of stunting malnutrition. Concerns regarding routine wide-spread antibiotic use include antimicrobial resistance and antibiotic-associated diarrhea. Antibiotic use from birth to age six months was associated with a 33% increased incidence of diarrhea (incidence rate ratio: 1.33, 95%CI: 1.12, 1.57) in children younger than three years old in India (192). In the meta-analyses presented in this thesis, antibiotics also had a more profound impact on weight gain than linear growth, and the antibiotic associated impact on linear growth was not statistically significant in the age group within the critical window of opportunity for intervention. Results of this subgroup analysis, however, did produce similar effect estimates to the full analysis, but may have been underpowered. Early antibiotic exposures (prior to age six months) has also been associated with an increased risk of childhood obesity in resource rich settings (193). The most appropriate target age for antibiotic use is therefore an additional consideration in order to maximize benefits and minimize future risks. The effect of different antibiotics may also vary, as has been found in observational studies of weight gain with antibiotic use in infants (325); although I was unable to evaluate impact of antibiotic class in my analyses.

Rogawski *et al.* (192) also found that in EBF infants, antibiotic use did not increase diarrhea incidence rates, suggesting that the rate of antibiotic-associated diarrhea after six months of age is modified by exclusive breast feeding until age six months. Given the high and early burden of enteric infections observed in many LMICs, Lang *et al*. recently suggested the 'judicious use of narrow-spectrum antibiotics to target specific pathogens'(190) and to limit antibiotic perturbation to the developing infant microbiota. Meta-regression analyses presented in this thesis did not find any difference in the antibiotic induced gains in height or weight between trials that used narrow- versus broad-spectrum antibiotics. This indicates that use of narrow-spectrum antibiotics could also produce clinically relevant improvements in child growth. A large RCT conducted in Malawi, found that community-based treatment of SAM with antibiotics, coupled with ready-to-use therapeutic food improved infant length and weight and reduced mortality risk (159). Careful consideration of antibiotic use, in combination with

interventions to promote EBF from birth to six months to reduce diarrhea risk and nutritional supplementation to ensure adequate nutrient intake, may be warranted. This may be particularly beneficial in populations with a high burden of HIV infection and SAM, where the treatment effect of antibiotics was greatest. However, further elucidation of potential risks to infants and the most appropriate infant age for antibiotic use are required before antibiotic use can be justified as a routine component of strategies to treat linear growth faltering in infants. More importantly, identifying the antibiotic induced changes in the gut microbiota that may contribute to infant growth, for example through improvements in nutrient absorption and energy harvesting, and clarifying the relative contributions of these changes versus alleviation of clinical or sub-clinical infections is essential in order to develop safer alternatives.

Two useful alternatives to antibiotics to promote healthy growth in infancy may be probiotic and prebiotic supplementation. In meta-analyses, infants less than 28 days of age fed probiotic supplemented formula for 120 days showed a significant increase in weight at the end of follow-up (1.5g/day, 95%CI:0.09 g, 2.93 g). The effect on height was not statistically significant, but this may have been due to the short follow-up period (20). Infants less than 28 days of age fed prebiotic supplemented formula also showed a significant increase in weight (1.07g/day, 95%CI: 0.14 g, 1.99 g) (21). An effect for height was not reported. Importantly, these meta-analyses reported no adverse effects of probiotic or prebiotic treatment in young infants. Conversely, an RCT of a combined probiotic and prebiotic supplement showed no benefit to child growth over ready-to-use therapeutic food (329). However, children in this trial were a median 22 months old (IQR: 15 to 32). By age 2-3 years, infants have already developed an adult-like gut microbiota. Interventions to promote healthy gut microbiota development need to be implemented earlier than was done in this RCT, in order to induce microbiota-mediated growth benefits.

This thesis also suggests a possible biological mechanism through which overgrowth of gut bacteria may impact linear growth in infants. Glutamate is an amino acid with an important role in amino acid metabolism, nitrogen balance and intestinal barrier function. An increase in the

abundance of *Acidaminococcus*, a genus of bacteria that can utilize glutamate as a sole source of carbon and energy, as well as an increase in genetic markers of the potential for bacterial fermentation of glutamate were both associated with future growth deficits between twins. The importance of glutamate to gut health and integrity is supported by *in vitro* studies and experimental studies using porcine models that have shown glutamate to improve epithelial barrier function. Biomarkers of EED and intestinal injury and repair (285) are associated with lower HAZ in LMICs. Impaired gut barrier function is also characteristic of EED, which is also associated with poor linear growth. Overgrowth of microbes in the gut that utilize glutamate as an energy source may result in lower glutamate availability for the infant, which may have a negative effect on gut barrier function, nutrient absorption and growth.

The findings of this thesis provide the first evidence of a relationship between linear infant growth and the abundance of gut microbiota members that may compete with the infant host for a limited micronutrient that is essential to gut health. The age of infants in these twin cohorts ranged from birth to 24 months at study entry, demonstrating that these growth associated microbiota changes occur during the time period associated with greatest risk of growth faltering. A broader implication of these findings is that micronutrient availability to the infant may, plausibly, be impacted by gut microbiota composition. Currently, the micronutrient with the strongest evidence base for use in promoting healthy linear growth is zinc. Evidence for an effect of other micronutrients is lacking. The impact of micronutrient supplementation could possibly be enhanced if implemented in combination with interventions to promote healthy gut microbiota composition and development during the critical period, such as prebiotics, probiotics and promotion of EBF to age six months. However, the mechanisms involved in microbiota associated growth faltering need to be confirmed before concrete recommendations can be made.

Limitations

In manuscript 1, the trajectory groups I identified may not be generalizable to infants outside of the ZVITAMBO cohort, and there is currently no optimal method to determine whether these

analyses identified the right number of growth trajectories. However, the cluster quality criterion that I utilized has been shown to perform well for finding the best number of clusters in a dataset. Rates of missing data were high for growth variables, as well as for a few key predictor variables. However, utilizing multiple imputations allowed all available subjects to be included in the analyses and reduced the risk of selection bias due to differential attrition and non-response. Key variables, such as access to clean water and sanitation were not available to assess as determinants of trajectory membership, and my analyses were unable to identify covariates that determine differences between the three closest infant trajectory groups.

In manuscript 2, the large degree of statistical heterogeneity between RCTs may limit the generalizability of the average antibiotic treatment effects on growth. The small number of trials limited the power to identify moderators of the treatment effect. It is, therefore, not completely clear which antibiotics, doses, or duration of treatment can be expected to produce these growth effects in other populations. However, pooling this diverse set of trials did allow identification of a few important sub-populations in whom the growth effect may be more profound. Antibiotic use may have a larger impact on growth in populations with high rates of HIV infection and malnutrition. Similar populations may benefit most from future, safer alternatives to antibiotics.

In manuscript 3, I could not control for confounding when comparing case and control network indices. These differences may, therefore, still be confounded by age or by other unreported factors that may impact microbiota composition and structure, such as weaning. However, the between-within multivariable regression models did control for unreported confounders that are shared between co-twins (e.g. fetal, maternal and environmental) as well as reported confounders that differ between siblings (e.g. diarrhea). However, residual confounding due to unreported factors that may differ between siblings, such as HIV status, remains a possibility. The small size of these cohorts and measurement error in the quantification of bacterial relative abundances also limited the power to detect changes in gut microbiota composition

associated with growth. Despite this, the microbiota associations with linear growth were reproduced in *both* cohorts; and the temporality in the relationships between changes in microbe abundance and growth deficits adds credibility to the results.

The studies in this thesis improve our understanding of linear growth faltering during the critical window from conception to 24 months of age, and point to areas for future research. The determinants of linear growth patterns suggest that infant growth may be predominantly determined by maternal characteristics and intrauterine growth. However exposures during infancy are also important. Interventions may be more effective if they begin during or prior to pregnancy and if implemented at an appropriate infant age. Understanding the mechanisms underlying the antibiotic growth-promoting effect in children will lead to safer second generation therapies for the treatment of child stunting. A possible mechanism through which infant gut microbiota composition may impact linear growth during the first thousand days may involve overgrowth of microbes that target important, limited, gut micronutrients for energy. Glutamate may be one such micronutrient. Clarifying these mechanisms will be important to inform optimal and safe approaches to achieving healthy growth in the world's population of vulnerable children.

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