The Gut Microbiome and Control of Weight Gain

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Introduction

Recognized as one of the most serious global health issues in our society, the prevalence of overweight and obesity in preschool children has increased over the last two decades to 6.7% in 2010 [1]. Obese children are more likely to be obese in adulthood and are at greater risk of adverse health outcomes in adult life and premature mortality [2]. The etiology of obesity is complex and involves lifestyle factors that are challenging to modify. Attention has therefore turned to preventative strategies and the identification of modifiable prenatal and early-life exposures associated with overweight risk in childhood.

Over the last decade, novel evidence from animal and human studies has identified associations between our intestinal bacteria (collectively known as our gut microbiota) and host metabolism and obesity [3–5]. Infancy is a critical period in the development of the commensal gut bacteria, with a gradual increase in colonization with the *Bacteroidetes* phylum from the time of birth. Initial colonization, especially with members of this phylum, is influenced by a number of early-life exposures including birth mode, infant nutrition, and antibiotic use [6, 7]. The introduction and wider use of next-generation sequencing techniques and metabolomic technologies have increased our ability to study gut microbiota, their metabolic functions, and associations with overweight.

This chapter summarizes current evidence on the link between infant gut microbiota and weight in children and discusses early-life interventions that impact its composition and may reduce future adiposity.

Link Between Gut Dysbiosis and Overweight

Obesity has been associated with alterations in the composition of intestinal bacteria, commonly known as gut dysbiosis. However, discrepancies exist in the nature and directionality of these shifts, some of which can be attributed to study design and microbial profiling methods. While experimental rodent models have provided important evidence regarding the link between gut microbiota and obesity, differences exist between animal model and human study design in microbiome research. These are comprehensively reviewed by Nguyen and colleagues [8] and only highlighted here. To start, there are dissimilarities in morphology between the human and murine gastrointestinal tracts. Unlike humans, in whom microbial fermentation of nondigestible dietary fiber takes place primarily in the proximal large colon, rodents

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have a well-developed cecum where fermentation occurs [8]. Lactobacilli comprise up to 25% of the murine gut microbiota, whereas in the human gut, they are mainly allochthonous (transient) members obtained from our diet [9]. In both humans and mice, the *Firmicutes* and *Bacteroidetes* dominate the intestine [10]. Yet, 85% of the bacterial taxa in cecal microbiota of mice represent genera that have not been detected in humans.

With major technological advancements in genomic sequencing over the last decade, our understanding of the human gut microbiome is rapidly and constantly expanding. Targeted qPCR (quantitative polymerase chain reaction) and culture methods, and even older molecular profiling methods (e.g., FISH cytometry), are being replaced with high-throughput genomic sequencing of whole microbial communities. This transition poses challenges for comparative evaluation across studies and synthesis of findings into theories of understanding. For example, targeted microbe studies make it difficult to assess if certain species are key obesogenic microbes or simply indicators of other aberrations in microbial taxa that have a greater influence on weight gain. On the other hand, genomic sequencing rarely is able to identify microbes at the species level. The reader is encouraged to peruse a user-friendly overview of current profiling methods in microbiome research by Tyler and colleagues [11].

Bacteroidetes and *Firmicutes*

The first evidence for an obesogenic gut microbiota profile implicated the phyla *Bacteroidete*s and *Firmicutes*. Ley and colleagues reported that obese, leptin-deficient *ob/ob* mice possessed reduced abundance of *Bacteroidetes* in their fecal samples and higher proportions of *Firmicutes* relative to their lean counterparts [10]. This higher ratio of *Firmicutes* to *Bacteroidetes* was later confirmed by Turnbaugh and coworkers in a study in a high-fat-diet-induced obese model [4, 12]. The high-fat diet intervention was associated with a bloom in a single clade of the *Firmicutes* phylum, *Mollicutes* [12], later reclassified as *Erysipelotrichaceae* [13]. In humanized gnotobiotic mice fed a high-fat diet to induce obesity, higher proportions of *Firmicutes* and *Erysipelotrichi* and a lower abundance of *Bacteroidetes* were found in stool samples [14]. Consistent with animal models, initial small-scale sequencing or qPCR studies in humans reported fewer microbiota in the *Bacteroidetes* phylum and a predominance of *Firmicutes* in the gut of obese versus normal-weight adults. In the study by Ley and coworkers, the abundance of *Bacteroidetes* increased with weight loss on a fat- or carbohydrate-restricted low-calorie diet [15].

The results of subsequent clinical investigations have been variably consistent with this paradigm. A systematic review and meta-analysis by Angelakis and coworkers [16] reported both lower and higher abundance of *Bacteroidetes* and a predominance of the *Firmicutes* phylum in the gut microbiota of obese/overweight adults. Likewise, Zhang and colleagues demonstrated that *Firmicutes* were dominant in obese adults compared to those who had undergone gastric bypass surgery; however, the *Prevotellaceae*, a family belonging to *Bacteroidetes*, were significantly enriched in obese subjects [17]. Conversely, Schwiertz and colleagues showed a reversal of the *Firmicutes* to *Bacteroidetes* ratios in obese individuals compared to lean controls; levels of the genus *Bacteroides* were higher, whereas numbers of clostridial clusters IV and XIVa, belonging to *Firmicutes*, were reduced in overweight or obesity [18]. Still others have not detected differences in genus *Bacteroides* between obese and lean subjects at baseline or after 8 weeks of a carbohydrate-restricted diet [19]. In this trial, statistical reduction was attained in the proportion of *Roseburia* and *Eubacterium*, members of the *Firmicutes* phylum, present in stool with successive decreases in total carbohydrate, starch, and non-starch polysaccharide intake.

The composition of gut bacterial species varies greatly between individuals [20], but microbial profiles are more similar among family members. Hence, monozygotic or dizygotic twins discordant for obesity provide an attractive model for studying associations between gut microbiota and obesity [21]. Using the twin design, Turnbaugh and colleagues observed low abundance of *Bacteroidetes* and *Actinobacteria* in obese individuals compared to their lean twins, but no significant differences in proportions of *Firmicutes* [5].

Lactobacillus and Bifidobacteria

The Waldram group found that genus *Bifidobacterium* was significantly less abundant in obese Zucker *fa/fa* rats compared to nonobese rats, in conjunction with significantly higher levels of the *Clostridium* cluster XIVa and *Lactobacillus* group [22]. Cani and colleagues also observed a reduction in *Bifidobacterium* and *Bacteroides* levels in mice fed a high-fat diet but less *Clostridium* cluster XIVa [23]. In the systematic review by Angelakis and coworkers [16], human 16S rRNA gene sequencing studies also reported lower concentrations of bifidobacteria but higher levels of lactobacilli in the gut microbiota of obese and overweight adults compared to lean individuals. Later studies by Million and colleagues found that some species of *Lactobacillus* (*L. reuteri*) were associated with obesity, while certain *Bifidobacterium* species were negatively correlated with body mass index (BMI) [24]. As further summarized by Koleva and colleagues [25], some *Lactobacillus* species promote weight gain to varying degrees (*L. ingluviei* > *L. fermentum* > *L. acidophilus*), while other species or strains cause weight loss (*L. plantarum*, *L. gasseri*) and are being tested for their effectiveness in overweight reduction.

Oscillospira and Akkermansia

Based on 16S rRNA gene surveys of the human microbiome, Konikoff and Gophna noted the association of an unculturable bacterium called *Oscillospira* with leanness or lower BMI in both infants and adults [26]. This association was supported by an elegant animal study comparing the microbiota response to fasting across five different vertebrate hosts, in which the abundance of *Oscillospira* increased after prolonged fasting in most animals [27]. Recently, Davis and colleagues reported an increase to the relative abundance of *Oscillospira* during weaning, especially after transition from breast milk to cow's milk [28]. It is unclear if the increase in *Oscillospora* is a consequence or a mediator of weight loss during caloric restriction. Another microbial species that inversely correlates with body weight in pregnant women and children is *Akkermansia muciniphila*; it is a well-known mucin-degrading bacterium that resides in the mucous layer of the gastrointestinal tract [29, 30].

Mechanisms in Microbially Induced Obesity

We are beginning to appreciate that human gut microbiota can contribute to obesity development in several ways. The majority of microbiota reside in the large intestine, where they produce short-chain fatty acids (SCFAs) from undigested carbohydrates, namely, dietary fiber and resistant starch, and to a limited extent from proteins. Elevated SCFA levels have been found in the colon of overweight adults and the serum of obese children [31, 32]. Total SCFAs are higher when *Firmicutes* microbes are prominent in adult stool and in children, especially for acetate, when the *Firmicutes* to *Bacteroidetes* abundance ratio is higher. The elevation of SCFAs is thought to result from excess production since SCFA absorption is not reduced in overweight versus lean individuals [32]. Once absorbed, SCFAs are used as energy for colonocytes or transported to various tissues such as the liver, where they are utilized in lipogenesis or gluconeogenesis. Excess fecal SCFA production by *Firmicutes* species in lean individuals has been equated with increased energy harvest and reduction in nutrient absorption. This caloric loss in stool is not evident in obese adult individuals, indicating enhanced energy extraction by gut microbiota in the overweight state [33].

Excess SCFA concentrations can stimulate colonic release of anorectic hormones, such as peptide tyrosine-tyrosine (PYY) and glucagon-like peptide (GLP)-1 [34]. These hormones also reduce colonic motility, which may enhance nutrient absorption and counter appetite suppression effects. As summarized in Table 4.1, other proposed mechanisms for gut microbiota involve intestinal permeability, systemic inflammation, and promotion of vagally mediated insulin and ghrelin secretion. No singular mechanism has been adequately studied in human adults or children, and many questions remain, most notably whether differences in gut microbiota and SCFA

Table 4.1 Mechanisms in microbially induced obesity

Research evidence	Study design/model
Biological pathway: gut microbiota influence energy harvest/storage via *pathways which break down dietary fiber into SCFA*	
*Germ-free mice have less total body fat than conventionally raised mice [3] *Conventionalization of germ-free mice leads to (a) 60% increase in total body fat and lower insulin sensitivity [3, 35], (b) increased ability of the host to extract energy from indigestible complex plant polysaccharides in the diet [3], and (c) higher ability of the host to regulate energy storage as triglycerides [35] *Transplanting microbiota from an obese twin to the nonobese twin mouse causes gains in total body and fat mass; obesity-associated metabolic phenotypes are also transmitted [36]	Germ-free mice; conventionalized (microbiota introduced through intestinal contents) germ-free mice; fecal transplantation in twin mice discordant for obesity
*In the leptin-deficient obesity model, bomb calorimetry shows that obese mice have less energy in their cecum than lean mice. The cecal contents of obese mice have a higher *Firmicutes* to *Bacteroidetes* ratio, are enriched with genes for enzymes that utilize nondigestible dietary carbohydrates to produce short-chain fatty acids (SCFAs), and have elevated concentrations of the SCFAs, butyrate and acetate [4]	Leptin-deficient obesity in mice
*When lean adults are fed a high calorie diet, a greater percent of ingested calories is found in their stool and thus not absorbed. The caloric content of stool is negatively correlated with a higher abundance of *Firmicutes* microbiota and a reduction in the *Bacteroidetes* [33]. Caloric loss is not evident in obese adults with comparable gut transit times to lean adults, suggesting that energy extraction by gut microbiota might be enhanced in overweight versus normal-weight individuals	Diet intervention in obese and lean adults
*Overweight children have much lower fecal concentrations of intermediate metabolites such as lactate yet higher levels of butyrate, a by-product of lactate-utilizing microbiota [37]. Their metabolite profile suggests exhaustive substrate utilization by obese gut microbiota. Rate of carbohydrate fermentation by gut microbiota has been found to be higher in obese vs. lean children and adolescents [31]	SCFA levels in obese and normal-weight children
Biological pathway: gut microbial SCFA metabolites influence nutrient intake, absorption, and utilization via *appetite hormones and colonic motility*	
*High-fat feeding alters the microbiome of rats, increasing the ratio of *Firmicutes* to *Bacteroidetes*. Changes in the microbiome lead to increases in production and turnover of acetate, which acts centrally to promote vagal activity, glucose-stimulated insulin secretion, hyperghrelinemia, and weight gain [38] *In both rodents and humans, direct colonic administration of the SCFA acetate increases blood levels of two gut hormones, glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) [34, 39, 40]. Primarily produced by enteroendocrine cells in the large intestine, these satiety (or anorectic) hormones diminish appetite [41]; lower PYY levels have been observed in overweight versus normal weight *Ileal/colonic infusions of acetate or a SCFA mixture into pigs/rats have also reduced gastric/colonic motility [39, 42]; the former has been attributed to concurrent PYY release *SCFA dose and site of action may be important. In van der Beek et al.'s study, distal colonic acetate infusions of adults, but not proximal colonic infusions, increased plasma PYY and fat oxidation [40]. In their human supplementation trial, Rahat-Rozenbloom et al. observed raised serum SCFA but not GLP-1 or PYY concentrations after a standard lunch and prior ingestion of dietary fiber [43]	Microbial acetate promotes weight gain in high-fat-fed rats SCFA or dietary intervention in humans and animal models
Biological pathway: gut microbiota influence intestinal permeability and systemic inflammation	
*Feeding mice a high-fat diet, Cani and coworkers observed elevated plasma LPS (lipopolysaccharide component of the cell wall of Gram-negative bacteria), weight gain, and insulin resistance [23]. The proportion of an LPS-containing microbiota also increased in the gut *This hypothesis is centered on the translocation of bacterial lipopolysaccharides (i.e., LPS) from the intestinal lumen to the circulation, which initiates systemic inflammation via activation of Toll-like receptors on macrophages [23, 44]	Diet-induced obesity in mice

in obese versus lean individuals are a cause or consequence of the obese state and if mechanistic insights from animal models can be extrapolated to humans. Finally, individual SCFAs may differ in their obesogenic potential or pathway. Comprehensive reviews of potential pathways for overweight that involve microbial metabolites can be found in review papers by Philip Gerard [44], Kumari and Kozyrskyj [45], and Rosenbaum and colleagues [46].

Origins of Gut Dysbiosis in the Development of Child Overweight

Gut microbial compositional differences with overweight are already evident in childhood. In Karlsson's case-control study of 4–5-year-old children, members of the *Enterobacteriaceae* family were overrepresented in fecal samples of overweight versus normal-weight children [29]. Cross-sectional studies by Bervoets and colleagues and Xu and colleagues have found higher *Firmicutes* to *Bacteroidetes* ratios in overweight versus normal-weight children [47, 48]. Bervoets and colleagues also reported *Bacteroides fragilis* to be more prevalent in gut microbiota of children with a higher BMI; however, other *Bacteroides* species such as *B. vulgatus* were less abundant, and lactobacilli were more prevalent in overweight children. In the Bervoets study, fecal concentrations of lactobacilli in children correlated with a serum marker of inflammation (C-reactive protein).

Normal Transitions in Gut Microbiota Development

It is likely that obesity-related changes in gut microbiota in children have their origins in infancy, at a time when the gut microbiome is established. Seeding of our gut microbiota begins at birth, and in some infants, it occurs in utero [49]. First colonizers, facultative anaerobes, lay the foundation for subsequent colonization by anaerobes of the *Actinobacteria* and *Bacteroidetes* phyla. New evidence from the GUSTO cohort shows that the higher initial presence of *Enterobacteriaceae* soon after birth predicts higher abundance of bifidobacteria in later infancy [50]. Throughout the first year of life, microbial diversity increases, converging toward the microbiota of the adult. Mode of delivery, infant diet, and maternal or infant antibiotic treatment are the main determinants of microbial colonization and development in infancy [6, 7]. The development of the gut microbiome during infancy plays a crucial role in the maturation of immunologic and metabolic pathways [51].

Abnormal Transitions that Precede Overweight

Indeed, compelling evidence supports the concept that shifts in the complex microbial system that occur early in life confer an increased risk for developing obesity. Indirect evidence for this thesis originates from studies of antibiotic use in infancy. Data from two large birth cohorts in Denmark and the UK found modest increases in risk for overweight at age 7 years and 38 months, respectively, with antibiotic treatment before 6 months of life [52, 53]. In a prescription database-linkage study, Azad and coworkers reported significantly greater odds of child overweight at age 9–12 years with exposure to antibiotics by age 1 but in male children only [54]. Their study also found an association between infant antibiotic treatment and central adiposity (measured by waist circumference), thought to be a better predictor of cardiovascular outcomes than BMI-based measures. A similar sex-specific effect of infant antibiotic treatment and BMI was reported from the International Study of Asthma and Allergies in Childhood [55].

To date, six epidemiological studies have published evidence on associations between infant gut microbiota and infant weight gain or later child overweight (Table 4.2). Two are nested case-control studies of children, matched according to birth mode, gestational age, birth weight, probiotic intervention group, breastfeeding duration, antibiotic use, and atopic disease status and

Table 4.2 Associations between gut microbial community and child overweight status

Authors and year of publication	Study design (location)	Participants (exclusion criteria)	Microbiota profiling time point and method	Overweight assessment	Main significant findings associated with overweight	Confounding variables considered in design/ analysis
Goffredo et al. 2016 [31]	Prospective cross-sectional study (US)	84 children and adolescents (use of medication known to affect liver function or alter glucose, lipid metabolism)	16S rRNA sequencing and qPCR	Body fat (total, visceral, sub cutaneous, hepatic) by fast magnetic resonance imaging, BMI	↑ *Firmicutes*-to-*Bacteroidetes* ratio ↑ Relative abundance of *Actinobacteria* and ↓ *Bacteroidetes* ↑ *Actinomyces*, *Bifidobacterium*, *Streptococcus*, *Blautia* ↓ *Odoribacter*, *Oscillospira*, *Bacteroides*, *Faecalibacterium*	Analysis adjusted for age, ethnicity, and gender
Dogra et al. 2015 [50]	Prospective general cohort (Singapore GUSTO cohort)	75 infants	Day 3, week 3, 3 months, and 6 months 16S rRNA sequencing	Subscapular skinfold thickness (mm) at 18 months	↑ *Streptococcus* abundances at month 6 Reaching more "mature" microbiota profile (high in *Bifidobacterium* and *Collinsella* and low in *Enterobacteriaceae*) later associated with ↓ skinfold thickness at 18 months	Analysis adjusted for gestational age and delivery mode
Scheepers et al. 2015 [56]	Prospective general and anthroposophic cohort (Dutch KOALA cohort)	909 infants (preterm birth before 37 weeks' gestation, twins, the presence of congenital abnormalities relating to growth, use of antibiotics before fecal collection)	1 month qPCR: bifidobacteria, *Bacteroides fragilis*, *Clostridium difficile*, *Escherichia coli*, lactobacilli, and total bacterial counts	Age- and gender-standardized BMI z-scores from parent's reported weight and height at 7 time points between ages 1 and 10 years	Positive *B. fragilis* colonization (*only* in children with low-fiber intake at age 4 years in conventional subcohort) ↑ *B. fragilis* counts in conventional high-fiber diet subcohort ↓ *B. fragilis* counts in low-fiber subcohort and alternative subcohort	Analysis controlled for gender, place and mode of delivery, birth weight, age at collection of fecal sample, maternal smoking during pregnancy, type of infant feeding in the first month, duration of breastfeeding, maternal education, and total bacterial counts

Bervoets et al. 2013 [47]	Prospective cross-sectional (Belgium)	26 overweight or obese and 27 normal-weight children	6–16 years qPCR: bacterial species belonging to the genera *Bacteroides*, *Bifidobacterium*, *Clostridium*, *Staphylococcus*, and *Lactobacillus*	Age- and gender-standardized BMI at age 6–16 years	↑ *Firmicutes*-to-*Bacteroidetes* ratio ↓ *B. vulgatus* ↑ *Lactobacillus* spp.	Age- and gender-standardized BMI
White et al. 2013 [57]	Prospective general cohort (Norwegian NOMIC cohort)	218 infants (preterm (GA < 253 days), term infants born via cesarean section, and term infants born vaginally but exposed to antibiotics before day 4 were excluded from the analysis)	4, 10, 30, 120 days BLAST: *Enterococcus* spp., *Lactobacillus* spp., *Lactobacillus paracasei/casei*, *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Lachnospiraceae* spp., *Veillonella* spp., *Pseudomonas* spp., *Escherichia coli*, *Enterobacteriaceae* other than *E.coli*, *Gammaproteobacteria*, *Varibaculum* spp., *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium* spp., *Bacteroides fragilis*, *Bacteroides* spp.	Difference in weight-for-age z-score from birth to 6 months from parent's reported weight	Negative *Bacteroides* spp. colonization at day 30 (males only)	Analysis controlled for antibiotic use after day 4, sex, use of milk substitutes, maternal smoking, and parity
Karlsson et al. 2012 [29]	Nested case-control (Sweden)	20 overweight or obese and 20 normal-weight children	4–5 years qPCR: *Lactobacillus*, *Bifidobacterium*, *Enterobacteriaceae*, *Akkermansia muciniphila*-like bacteria, *Desulfovibrio*, and *Bacteroides fragilis*	Age- and gender-standardized BMI at age 4–5 years	↑ *Enterobacteriaceae* ↓ *Desulfovibrio* ↓ *Akkermansia muciniphila*-like bacteria	Age- and gender-standardized BMI
Xu et al. 2012 [48]	Case-control (China)	85 overweight/obese and 91 normal weight (antibiotic administration within 2 weeks of fecal sample, stress, gastrointestinal disorder, polio vaccination within 1 month)	7–13 years qPCR: *Bacteroidetes*, *Firmicutes*	Age- and gender-standardized BMI	↑ *Firmicutes*-to-*Bacteroidetes* ratio ↓ *Bacteroidetes*	Age- and gender-standardized BMI

(continued)

Table 4.2 (continued)

Authors and year of publication	Study design (location)	Participants (exclusion criteria)	Microbiota profiling time point and method	Overweight assessment	Main significant findings associated with overweight	Confounding variables considered in design/ analysis
Luoto et al. 2011 [58]	Nested matched case- control (Finland)	15 overweight or obese and 15 normal-weight children with family history of atopic disease	3 months FISH: *Bacteroides-Prevotella* group, *Bifidobacterium* genus, *Clostridium histolyticum* group, *Lactobacillus-Lactococcus-Enterococcus* group, and total counts	BMI at 10 years from parent's reported weight and height	↓ Bifidobacteria numbers (NS)	Matched for sex, gestational age, BMI at birth, mode of delivery, probiotic intervention, and duration of breastfeeding
Vael et al. 2011 [59]	Prospective general cohort (Belgium)	138 infants (preterm birth, delivery by cesarean section)	3, 26, and 52 weeks Cultures: *Bacteroides fragilis*, *Bifidobacterium*, *Lactobacillus*, *Enterococci*, *Enterobacteriaceae*, *Clostridium*, *Staphylococcus*	BMI at 12, 18, 24, 30, and 36 months from parent's reported weight and height	↑ *B. fragilis* concentration at 3 and 26 weeks ↓ *Staphylococcus* concentration at 3 and 52 weeks ↓ *Staphylococcus/B. fragilis* ratio at 3 weeks	Analysis controlled for maternal BMI, formula or breastfeeding, antibiotic use in infancy, SES, maternal smoking status, birth weight
Kalliomaki et al. 2008 [60]	Nested matched case- control (Finland)	25 overweight or obese and 24 normal-weight children with family history of atopic disease	6 and 12 months FISH/FISH-FCM: *Bacteroides-Prevotella* group, *Bifidobacterium* genus, *Clostridium histolyticum* group, *Lactobacillus-Lactococcus-Enterococcus* group, and total counts qPCR: *Bifidobacterium* genus, *Bifidobacterium adolescentis*, *Bifidobacterium bifidum*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Bacteroides fragilis*, *Staphylococcus aureas*	BMI at 7 years from parent's reported weight and height	↓ Bifidobacterial numbers ↑ *S. aureus* numbers	Infants matched for gestational age, BMI at birth, mode of delivery, probiotic intervention, duration of breastfeeding, antibiotics in infancy, and frequency of atopic diseases and sensitization at 7 years of age

BLAST basic local alignment search tool, *BMI* body mass index, *FISH-FCM* fluorescent in situ hybridization coupled with flow cytometry, *qPCR* quantitative polymerase chain reaction, *NS* nonsignificant, *SES* socioeconomic status

selected from a prospective follow-up of high-risk (for allergy) infants [58, 60] randomized to pre- and postnatal probiotic supplementation [61]. Using FISH flow cytometry and qPCR methods, Kalliomaki and coworkers reported lower bifidobacterial numbers and higher counts of *Staphylococcus aureus* in fecal samples obtained at 6–12 months after birth in 7-year-old children classified as overweight versus normal weight [60]. At 6 months of age, there was a trend for lower counts of lactobacilli but higher counts of *B. fragilis* in the children who became overweight. In a follow-up study at age 10, Luoto and coworkers found that fecal bifidobacteria also tended to be lower in number in 3-month-old infants who developed overweight compared to those who did not [58].

In a general population cohort of vaginally delivered full-term infants, higher *B. fragilis* in gut microbiota at age 3–26 weeks and lower staphylococcal concentrations (as measured by culture) were correlated with a higher BMI z-score in preschool children between 1 and 3 years of age [59]. Analyses were adjusted for known risk factors of childhood overweight, including maternal BMI and smoking status, birth weight, breastfeeding status, and infant use of antibiotics. On the other hand, a prospective follow-up of full-term infants, delivered vaginally and not exposed to antibiotics, found early detection of *Bacteroides* species (as per DNA cloning methods) in fecal samples at 1 month of age to be associated with a reduced growth trajectory over the first 6 months of life [57]. This was observed in male infants only; the presence of *Staphylococcus* species at day 4 was associated with expected growth in both males and females. Findings were independent of maternal BMI and other pregnancy complications, fetal growth, birth weight, and breastfeeding status.

Additional evidence for a relationship between gut microbial composition and infant weight gain comes from the large prospective KOALA Dutch birth cohort study [56] of offspring from women following a conventional or anthroposophic (alternative) lifestyle (based on dietary habits, child-rearing practices, vaccination schemes, and/or use of antibiotics). All results were adjusted for several confounding factors, including prepregnancy overweight, birth mode, breastfeeding duration, and caloric intake at age 4. In the conventional cohort, newborn fecal colonization with *B. fragilis* at 1 month postpartum was associated with a higher BMI z-score until age 10 but only among children with a low-fiber intake at age 4. Among newborns who were colonized with this microbe, *B. fragilis* counts were positively correlated with BMI z-score in children eating a high-fiber diet in the conventional cohort and were negatively correlated with future BMI in the low-fiber and anthroposophic cohorts. Newborn colonization with *C. difficile* at 1 month in the conventional cohort was associated with lower BMI z-score at 8 ½ years of age. The *C. difficile* finding will be discussed further in the context of breastfeeding and weaning in the next section.

Another recent prospective cohort study from Singapore (GUSTO) reported on microbiota acquisition from birth to 6 months of age in relation to delivery mode and gestational age, as well as associations with later adiposity [50]. This study found that infants who acquired a profile high in *Bifidobacterium* and *Collinsella* (of the *Actinobacteria*) and low in *Enterobacteriaceae* at 6 months versus earlier (from 3 days to 3 months after birth) had lower adiposity, as measured by subscapular skinfold thickness, at 18 months of age. A linear association between *Streptococcus* abundance at month 6 and changes in subscapular skinfold thickness from birth to 18 months was also observed. Both findings were independent of gestational age and delivery mode.

In sum, there is a relative dearth of prospective studies testing the association between gut microbiota in infancy or childhood and subsequent overweight. Studies by Vael and coworkers, Bervoets and coworkers, and Scheepers and coworkers, as well as other human adult investigations, point to a role for *Bacteroides* spp. in weight control in early life [15, 47, 56, 59, 62]. Gut lactobacilli, bifidobacteria, staphylococci, streptococci, and enterobacteria may also be important for regulating growth in infants and young children. It is also likely that growth is sensitive to perturbations in diet or other environ-

mental factors during critical windows of microbiota development. For example, Vael and coworkers and White and coworkers reported stronger correlations between infant growth and *B. fragilis* counts at <26 weeks but not later during infancy [57, 59].

Breast Milk Intervention to Alter Gut Microbiota and Prevent Obesity

Since the gut microbial community is strongly implicated in weight control, manipulating this "organ system" has the potential to prevent or treat obesity. Intervening during infancy offers new therapeutic possibilities. As exciting as this intervention appears, fecal transplantation has been primarily tested in animal models and in humans, only in adults with *C. difficile* diarrhea [63]. Hence, we have focused our discussion on the manipulation of gut microbiota by human milk, a natural modifier of gut microbial composition. For a full review on the effectiveness of probiotics and prebiotics in reducing excessive weight gain in children, see Koleva and coworkers [25].

Breast Milk Feeding

Uniquely adapted to infants to provide complete nutrition during the first 6 months of life, human milk has been associated with a number of health benefits, including reduced risk of later overweight [64]. Human milk contains a large proportion of bioactive compounds important in the stimulation of the immune system and intestinal microbiota [65, 66]. Human milk oligosaccharides (HMO) represent the third largest component of human milk [67]. They are complex sugars that resist digestion by the stomach and reach the small intestine and colon intact where they are metabolized by selective intestinal microbiota, increasing their numbers and function within the gut [68]. HMO metabolism leads to the production of short-chain fatty acids (SCFAs), which reduce the pH of the intestinal lumen, alter microbial composition, and inhibit pathogen growth [69]. As discussed in Table 4.1, SCFA may contribute to dietary energy harvest, modulate host adiposity, and alter gene expression of host satiety hormones.

In addition to providing substrates for microbial metabolism, there is evidence from several studies demonstrating the presence of live bacteria in human milk [70]. Summarized by McGuire and McGuire, the large diversity and richness of the human milk microbiome include, but is not limited to, *Bifidobacterium*, *Lactobacillus*, *Staphylococcus*, and *Streptococcus* [70]. The presence of bacteria in human milk was thought to be a result of contamination from maternal skin. However, this has been disputed by studies which show that orally administered probiotics given to lactating women can be detected in human milk [71, 72] and knowledge that certain milk genera, such as bifidobacteria, are strict anaerobes.

Accordingly, differences in early gut microbiota between breastfed and formula-fed infants have been observed in several studies. Using targeted qPCR techniques, Penders and colleagues found exclusively formula-fed infants at 1 month of age to be colonized with *E. coli*, *C. difficile*, *Bacteroides*, and lactobacilli to a greater extent than breastfed infants [7]. With 16S rRNA gene sequencing and targeted qPCR, Azad and colleagues characterized the gut microbiota of non-breastfed infants as having higher species richness at 3 months, with overrepresentation of genus *Akkermansia* and *C. difficile* [6]. Of note, 20–60% of breastfed infants were colonized with *C. difficile*. In a large sample from the same Canadian cohort, breastfeeding exclusively at 3 months was inversely associated with *Bacteroidetes* and *Clostridiales*, including *Veillonellaceae*, *Lachnospiraceae*, and *Ruminococcaceae* [73]. Breastfeeding also increases levels of fecal immunoglobulin A (IgA) in infants at 3 months of age in a dose-dependent manner [74].

Weaning off breast milk has been reported to have a greater impact on infant gut microbial composition than other early-life exposures [75]. In the systematic review by Vail and colleagues, ten observational studies found an inverse associ-

ation between age at weaning and infant growth, but reverse causation was a likely explanation in four studies [76]. The introduction of cow's milk, even in small quantities while breastfeeding [28], has been associated with dramatic increases in the abundance of genera *Bacteroides* and *Oscillospira* and the disappearance of *C. difficile* colonization [28].

Breastfeeding and Maternal Prepregnancy Overweight, Pregnancy Weight Gain, Birth Mode, and Probiotic Use

Human milk is not uniform and can differ significantly between mothers [77], including the presence of specific microbial taxa [70]. While some of this variation may be a function of methodological issues, recent studies have shown that certain maternal factors, including weight status, birth mode, and probiotic use, can influence the composition of human milk and infant gut microbiota. Herein, we address a number of commonly asked questions about the relationships among breastfeeding, the microbiome, and childhood weight gain.

Is Overweight in a Breastfeeding Mother Associated with Changes in Infant Gut Microbiota?

Strong evidence exists that maternal pregnancy overweight is a risk factor for overweight in offspring [78] and that breastfeeding can lower this risk [64]. Pregnancy overweight has also been associated with changes in both breast milk and infant gut microbiota. Characterizing breast milk microbiota [79], Cabrera-Rubio and colleagues observed higher quantities of genus *Staphylococcus* and less *Bifidobacterium* in human milk of obese compared to normal-weight Finnish mothers over the first 6 months of breastfeeding. *Lactobacillus* was dominant in the colostrum (first breast milk) and in mature breast milk at 6 months. Excessive pregnancy weight gain was associated with similar compositional patterns of breast milk. However, the gut microbiota of their infants was more likely to be colonized with *C. difficile* at 6 months of age and to have lower total bifidobacterial counts. In a larger sample of infants from the same cohort ($n = 42$), who were exclusively breastfed for 3–4 months on average [80], prepregnancy overweight was associated with lower counts of genus *Bacteroides* in the infant gut at 1 month, but not at 6 months of age. Instead, at this later age, prepregnancy overweight was associated with a greater likelihood of gut colonization with *C. difficile* and *Akkermansia muciniphila* and higher counts of staphylococci, but lower concentrations of bifidobacteria.

As shown in the Santacruz and colleagues' study of Spanish women, maternal microbial influences on offspring weight are already evident at birth [30]. High birth weight following prepregnancy overweight or excessive weight gain during pregnancy was associated with a maternal fecal microbiota enriched with *E. coli* and depleted in lactobacilli. In a Finnish cohort, Cabrera-Rubio and colleagues detected fewer bifidobacteria in third-trimester fecal microbiota of women with prepregnancy overweight [79]. Maternal pregnancy weight influences may be reinforced by the microbial composition of breast milk soon after birth, which becomes enriched with staphylococci when levels of bifidobacteria are low, independent of gestational age and delivery mode [81].

Alongside modifications to milk microbiota in overweight women seen for the first 6 months of breastfeeding, dysbiosis of the gut microbiome is observed in their infants. Gut dysbiosis soon after birth is likely the product of cesarean delivery, common with overweight mothers. Early *C. difficile* colonization of the infant gut following pregnancy overweight could simply be an indicator that the infant is being breastfed since *C. difficile* levels drop abruptly after weaning to cow's milk [28]. Gut microbial changes which emerge later in infancy, such as reductions in the counts of bifidobacteria or staphylococci, may indeed, be promoted by breast milk composition in overweight mothers. There is also the possibility that these changes to gut microbiota predate the initiation of breastfeeding.

Will Breastfeeding After Cesarean Section Delivery Reverse Infant Gut Dysbiosis and Reduce Risk for Overweight?

Cesarean section is associated with changes in gut microbiota beginning soon after birth, as indicated by dramatic reductions in abundance of *Bacteroidetes* at 3 months [73]. The literature is divided, however, on whether child overweight development can be attributed to this surgical intervention [78]. At issue is that both types of cesarean deliveries, elective and emergency, are often combined in published analyses. Interestingly, exclusive breastfeeding 3 months after birth does not contemporaneously alter cesarean-induced microbial changes in the gut but is associated with future resolution of dysbiosis at 1 year of age [73]. This phenomenon is seen primarily in infants delivered by emergency cesarean. On the other hand, mothers giving birth by elective cesarean section possess breast milk microbial profiles which are distinct in composition from those found after emergency cesarean or vaginal birth [79].

Limited evidence suggests that breastfeeding can reverse cesarean-induced changes in the infant gut microbiome, but it is up for debate whether this effect alters risk for overweight.

Do Maternal Probiotics Taken While Breastfeeding Reduce Risk for Overweight?

Depending on the strain and species, *Lactobacillus* has weight-promoting activities; yet this genus has been shown to reduce excessive weight gain in infants when administered to the mother prenatally [24]. Inconsistent weight gain and loss effects have been reported for *Lactobacillus reuteri* across studies [82]. In the randomized controlled trial by Abrahamsson and colleagues [83], unexpected variations in gut microbial composition of breastfed infants were found following maternal treatment with the probiotic, *L. reuteri.* Viewed as evidence for the transmission of the probiotic to breast milk, *L. reuteri* was detected in maternal colostrum; other species of *Lactobacillus* were also elevated in colostrum. Yet, the prevalence of *L. reuteri* declined in breast milk and newborn gut microbiota after the first week of continuous supplementation. Moreover, despite being detected in breast milk, *L. reuteri* levels were lower in the gut microbiota of infants breastfed than those formula-fed. This reduction of *L. reuteri* was interpreted as the outcome of competition from other microbiota and/or immune recognition of *L. reuteri* by immunoglobulin A found in mother's milk.

To conclude, the effectiveness of probiotic treatment in weight control is specific at the species and strain level; maternal probiotic intake while breastfeeding is found to have unpredictable effects on infant gut microbiota.

Conclusions and Future Perspectives

Advances in gene sequencing technologies have yielded considerable evidence for the role of gut microbiota in the control of weight gain. As tempting as it is to proceed to translation into practice, inconsistencies between animal experimentation and human observation must be reconciled. We have also alerted the reader to the fact that gut microbial compositional and metabolite biomarkers for obesity in adulthood are not applicable to infancy, a period of substantial plasticity when gut microbiota are being shaped by early-life exposures. Evidence from studies in adults and children may, in fact, impede understanding of the mediating role of early-life gut microbiota in controlling weight gain. It is imperative that hypotheses that emerge from animal models and human adult studies be verified in human infants. Equally, prospective follow-up studies of birth cohorts are needed to provide unbiased signals of microbiome-health associations for further testing in animals.

Regarding birth cohort studies, the potential for bias remains if analytical strategies do not take into account important confounding factors, such as birth mode, breastfeeding status,

and antibiotic use. Bias arising from study differences in design (prospective vs. nested case-control), selection of subjects (general population vs. high risk for atopy), and variable time points for infant fecal sampling and overweight assessment can also lead to conflicting results. Large, longitudinal cohort studies that employ gene sequencing to profile the whole gut microbial community in fecal samples obtained at age-sensitive time points, and which collect detailed information on early and also later childhood covariates such as diet and level of activity, are required to enhance understanding of how perturbations in infant gut microbiota can lead to overweight.

The effectiveness of breastfeeding, as a dietary intervention discussed in this review, is dependent on the stage of gut microbiota development, complementary diet, and health status of the mother. As we pointed out, women with prepregnancy overweight have altered breast milk composition, and their infants show early, transient, and later changes to gut microbial community structure. Breast milk itself may interact with the host system of the infant to modify the effectiveness of administered probiotics. Timing of the intervention is an important consideration. With the detection of microbes in the placenta and amniotic fluid, and in meconium (infant's first stool) [49], development of gut microbiota has been extended to the time of pregnancy and now subject to maternal influence.

Finally, keystone microbial species or metabolites are potentially too simplistic as biomarkers for overweight and metabolic disorders and require testing and refinement. Microbial SCFAs have been implicated in the development of overweight in humans as well as rodents, though the relative production of acetate and other SCFAs by specific microbes may vary depending on the pH of the large intestine and substrate availability [45]. As new theories emerge on metabolic pathways to overweight, they will guide our search for gut microbial biomarkers. Finally, as suggested by reports of sex differences in gut microbiota and infant risk for overweight [54], new theories may need to consider the influences of infant sex, ethnicity, and geographic location on breastfeeding and pre-/probiotic interventions aimed at preventing childhood obesity.

Editor's Questions

Is it possible that the effects of the microbiome on childhood growth, weight gain, and metabolic function depend upon the complex and changing interactions of many (or all) members of the "microbial community" (including viruses and fungi) rather than on the contributions of single classes of microbes? If so, does this make supplementation of single class or species of microbes less likely to exert dramatic effects on body habitus and metabolic phenotype?

Authors' Responses

Ecosystem interactions among all microorganisms (including viruses and fungi) resident in and transient to the gut are likely more complex than our current knowledge indicates. Microbes constitute the largest metabolic potential within this community and their SCFA metabolites have been implicated in overweight. Many gut microbiota produce the same SCFA (i.e. acetate). While other SCFAs are preferentially produced by specific microbes (e.g., propionate synthesis by members of the *Bacteroidetes* phylum), even these SCFAs can be produced by alternate microbiota depending on the pH of the large intestine and substrate availability (sugars, lactate, proteins, fats). In view of the above, supplementation with a single species of microbes is unlikely to produce anticipated effects.[a]

Reference for Authors' Response Section

(a) Kumari M, Kozyrskyj AL. Gut microbial metabolism defines host metabolism: an emerging perspective in obesity and allergic inflammation. Obes Rev. 2017;18(1):18–31.

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