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Themed Issue: The Gut Microbiome and Immunity: How it is Shaped by Early Life

REVIEW

Fetal programming of overweight through the microbiome: boys are disproportionately affected

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Maternal and childhood obesity in pregnancy are worrisome public health issues facing our world today. New gene sequencing methods have advanced our knowledge of the disruptive effect of birth interventions and postnatal exposures on the maturation of gut microbiota and immunity during infancy. Yet, little is known about the impact of maternal pregnancy overweight on gut microbes and related processes, and how this may affect overweight risk in offspring. To address this gap in knowledge, we surveyed human studies for evidence in children, infants and pregnant women to piece together the limited literature and generate hypotheses for future investigation. From this literature, we learned that higher *Lactobacillus* yet lower *Bacteroides spp.* colonization of gut microbiota within 3 months of birth predicted risk for infant and child overweight. The abundance of bifidobacteria and staphylococci also appeared to play a role in the association with overweight, as did infant fecal immunoglobulin A levels, glycoproteins of the gut immune system that are acquired from breast milk and produced by the infant. We proposed that pregnancy overweight influences the compositional structure of gut microbiota in infants through vertical transfer of microbiota and/or their metabolites during pregnancy, delivery and breastfeeding. Finally, we brought forward emerging evidence on sex dimorphism, as well as ethnic and geographic variation, in reported associations between maternal overweight-induced gut microbiota dysbiosis and overweight risk.

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Introduction

Nutrition and other environmental stimuli or insults, which occur during critical periods of developmental plasticity, change the capacity of the host to cope with future environmental pressures and increase susceptibility to disease. Known as the developmental origins of health and disease (DOHaD), this theory has motivated research into the fetal origins of adult disorders over the last 30 years.[1] The DOHaD approach has led to many advances to our knowledge on how the fetus adapts to a wide range of environmental exposures, including undernutrition, stress and more recently over-nutrition. Originally coined by Barker as the 'fetal origins' hypothesis, based on observed associations between low birth weight and adult cardiovascular disease,[2] the notion of birth weight as a causal marker of future disease has been challenged due to physiologic factors such as maternal size, and the strong role of the postnatal environment in human development. Outnumbering human cells 10 to 1 when fully mature, our gut microbiome of commensal microbes is an 'organ' system, which is heavily shaped by the postnatal environment in concert with the developing immune system.

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With advances in gene sequencing technology, we are better able to characterize the gut microbiome, this complex ecosystem of 500–1000 microbial species and their genes that is essential for host absorption of nutrients and protection against pathogens. Hence, this previously understudied gut microbial 'organ' is now under intense scrutiny. Our group has previously published on the infant gut microbiome, presenting evidence for its postnatal programming role in the origins of childhood disease.[3] In this review, we focus on *fetal* programming of the early infant gut microbiome by maternal overweight and its subsequent impact on child overweight.

Childhood overweight can be linked to prenatal exposures

Childhood obesity is one of the most worrisome public health issues in the world today. In the United States, overweight in early childhood has risen to 27% in the past 20 years.[4] Over 20% of preschool children in Canada are overweight.[5] Overweight children are at higher risk of remaining overweight in later life and developing insulin resistance, metabolic syndrome and type 2 diabetes.[6,7] To date, much of the research on the origins of childhood overweight has centered on dietary excesses and inactivity, and on identifying obesogenic genes.

Challenges in treating overweight in childhood highlight gaps in current understanding of what underlies the initiation and persistence of this state. For this reason, recent attention

has turned to the pre- and postnatal time periods. Maternal overweight during pregnancy has been identified as an early life risk factor for child overweight, in addition to other postnatal factors including low and high birth weight, rapid infant weight gain and limited breastfeeding.[7] Affecting between 30–50% of pregnancies in Canada and the United States,[8,9] and in some US populations close to 70% of pregnancies,[10] maternal overweight during pregnancy has also become a public health concern. Pregnancy overweight has been linked to a myriad of pregnancy complications, such as vitamin D insufficiency, gestational diabetes and cesarean delivery.[11,12]

Infant gut microbiota and metabolites as mediators of overweight

Landmark studies in mice[13] and in human adults have reported divergent gut microbiota patterns and short-chain fatty acid (SCFA) metabolite profiles between obese and lean subjects.[14] Meta-analytic evidence from these studies suggests that an altered balance of the two dominant intestinal (gut) bacterial phyla, Bacteroidetes and Firmicutes, may be obesogenic.[14] Gut microbes ferment indigestible foods to SCFAs, butyrate, propionate and acetate, making them available to gut epithelial cells as an energy substrate.[15] Produced chiefly by members of the Clostridia class (of the Firmicutes phylum), butyrate is a key energy source.[16] Two studies, one in overweight adults and the other in germ-free mice reconstituted with microbiota from overweight adults, reported obesity to be associated with higher abundance of *Bacteroides* and lower production of propionate and butyrate.[17,18] Lactobacilli are also more common in the gut microbiota of obese adults.[19] As summarized by Koleva *et al.*,[20] *Lactobacillus* species promote weight gain to varying degrees, while some species or strains cause weight loss and are being tested for their effectiveness in overweight reduction.

Compositional differences in gut microbiota between overweight and normal weight are already evident in childhood.[21,22] In Karlsson's case–control study of 4–5-year-olds, members of the *Enterobacteriaceae* family were more often identified in fecal samples of overweight *v.* normal weight children.[21,23] Bervoets *et al.*'s[22] cross-sectional study of older children (age 6–10 years) reported *Bacteroides fragilis* to be more prevalent in gut microbiota of children with a higher body mass index (BMI). Payne *et al.*[24] found overweight children to have much lower levels of metabolites such as lactate (produced by lactic acid bacteria), yet higher levels of butyrate than normal weight children at age 8–14 years. However, differences observed in adults and children may not apply to young infants, at a time when gut microbiota are being shaped by infant diet and prior prenatal and birth events.[25,26] Longitudinal studies beginning early in gut microbiota development are needed to confirm if dysbiosis of human gut microbiota causes overweight or is simply a result of it.[27]

Of the limited number of studies in children to date,[21,28–31] four have shown that microbiota dysbiosis, as early as 3–6 months of age, preceded development of childhood overweight.[28–31] Kalliomaki *et al.*[30] reported lower bifidobacterial numbers and higher counts of *Staphylococcus aureas* in fecal samples at 6 and 12 months in overweight children than those of normal weight at age 7 years. Overweight children had lower counts of lactobacilli but higher counts of *B. fragilis* at 6 months of age. In a smaller sample of children from the same study, Luoto *et al.*[28] found that bifidobacterial numbers also tended to be lower in fecal samples at 3 months of age in children who developed overweight at age 10 compared with those who did not. In a general population birth cohort study of vaginally delivered full-term infants, higher *B. fragilis* in gut microbiota at age 3–26 weeks and lower staphylococcal concentrations were correlated with a higher BMI *z*-score in preschool children.[29] Regression analyses controlled for many known risk factors of childhood overweight, including maternal BMI and smoking status, breastfeeding status and infant use of antibiotics. On the other hand, a prospective follow-up of full-term infants by White *et al.*,[32] delivered vaginally and not exposed to antibiotics, found early colonization with *Bacteriodes* species at 1 month of age to be associated with a reduced growth trajectory over the first 6 months of life in male infants. Findings were independent of maternal BMI and other pregnancy complications, fetal growth and birth weight, and breastfeeding status.

To add to this evidence are new findings on intestinal microbiota composition and infant weight gain from the large KOALA Dutch birth cohort study.[31] Gut colonization with *B. fragilis* at 1 month postpartum was significantly associated with a higher BMI *z*-score, measured repeatedly between 1 and 10 years, but only among infants with a low fiber intake at age 4. Among colonized children, *B. fragilis* counts were positively associated with BMI *z*-score in children in a high-fiber diet subcohort and negatively associated with BMI in a low fiber subcohort. All analyses were adjusted for pre-pregnancy overweight, birth mode, breastfeeding duration, caloric intake at age 4 and many other covariates. Linear regression analysis also revealed that infants colonized with *Clostridium difficile* at 1 month had a lower BMI *z*-score at $8\frac{1}{2}$ years of age.

In summary, overweight has been associated with higher abundance of genus *Bacteroides* in the intestinal microbiota of adults and children. In infants, however, it appears that *Bacteroides* spp. colonization of the gut 1 month after birth can reduce growth, and that positive associations between early colonization or counts of genus *Bacteroides* and BMI during childhood are influenced by the fiber content of the preschool diet. Several of the earlier longitudinal studies of the infant gut microbiome were nested case–control studies of children, matched on birth mode, infant feeding characteristics and atopic disease status, who had been selected from a prospective follow-up of high risk (for allergy) infants from randomized probiotic supplementation trials. Newer studies represent the prospective follow-up of fecal samples of infants born in general population birth cohorts. Details on study design, sample size, fecal sequencing methods and other findings have been presented in our recent review paper on the gut microbiome and obesity risks.[33]

Critical windows for infant development of gut microbiota and immunity

The first colonizers of infant gut microbiota lay the foundation for subsequent colonization with anaerobes from the Clostridia and Bacteroidia classes.[34–37] Historical depictions of the fetal intestine as sterile have been challenged by recent studies that detect microbes in the placenta and amniotic fluid,[38] as well as in meconium, the infant's first stool.[39] In a study of 20 term newborns, Gosalbes *et al.* reported two distinct profiles of meconium microbiota composition; meconium dominated by lactic acid bacteria was found more often following the consumption of organic food during pregnancy.[40] They also noted that fecal samples at 7 months of age contained microbial species originally found in meconium.

The innate immune system and production of pro-inflammatory cytokines evolves in early infancy in parallel to that of the gut microbiota.[41] A fecal marker of gut mucosal immunity is secretory immunoglobulin A (IgA), a glycoprotein, which participates in the innate and adaptive immune systems to inhibit penetration of pathogens, and prevent local inflammation to gut microbiota and innocuous antigens.[42] Murine studies that experimentally suppress IgA document elevated levels of gut *Enterobacteriaceae* and reduced *Bacteroidaceae*.[43] Initially provided by breast milk, an infant's own ability to produce IgA in B cells in the gut epithelium commences in the first few weeks after birth and increases throughout infancy.[44] Higher secretory IgA levels are evident at 6 months after colonization with lactobacilli at 1 month of age;[45] increases to infant fecal IgA levels have been observed after the administration of a *Lactobacillus rhamnosus* and *Bifidobacterium breve* probiotic mixture to pregnant women and their newborns.[46] Lower salivary secretory IgA has been reported in overweight children.[47] Of note, epithelial cells extracted from meconium are able to express IgA.[48] Hence, studies of meconium may provide new clues on the maturation of gut microbiota and immunity, and how both are shaped by the fetal environment.

Role of the maternal pre- and postnatal microbiome in obesity risk of offspring

Alongside scrutiny of the literature to determine whether associations between maternal and offspring overweight indeed have a fetal origin,[49] hypotheses are continuously being put forward regarding pathways of influence. In the review by Paliy *et al.*, several prenatal and postnatal hypotheses were considered in relation to maternal diet and breast milk composition, as well as infant gut microbiota changes.[50] Cox *et al.* provided a comprehensive overview of microbe-dependent mechanisms for overweight via increased energy harvest from, and altered metabolic signaling and inflammation of gut microbiota in infants.[51] Both reviews also noted that microbe-induced obesity can involve events such as cesarean section delivery and maternal antibiotic exposure, which disrupt the transfer of maternal microbes during pregnancy and birth. Overweight during pregnancy increases the likelihood of cesarean section delivery, and treatment with antibiotics during pregnancy and delivery.[12,52] It also increases the risk for neonatal group B streptococcal infection and treatment of newborns with intravenous antibiotics.[52,53] As summarized in Fig. 1, this and the next section will address hypotheses related to microbiota-induced obesity as a consequence of fetal exposure to maternal overweight.

From the landmark study by Koren *et al.*,[54] it is evident that the gut microbiome of all women undergoes dramatic compositional changes during pregnancy. Using a culture independent approach, this study compared the gut microbial communities of pregnant women in their first and third trimesters. Significant shifts in microbiota diversity occurred in all women and were unrelated to pre-pregnancy BMI, gestational diabetes or use of antibiotics during pregnancy. These shifts included a reduction in the total number of bacterial taxa in the third trimester ($P < 0.0001$); at the same time, the relative abundance of lactic acid bacteria (lactobacilli, streptococci, enterococci), Proteobacteria, specifically the *Enterobacteriaceae* ($P < 0.0004$), and members of the Actinobacteria ($P < 0.003$)

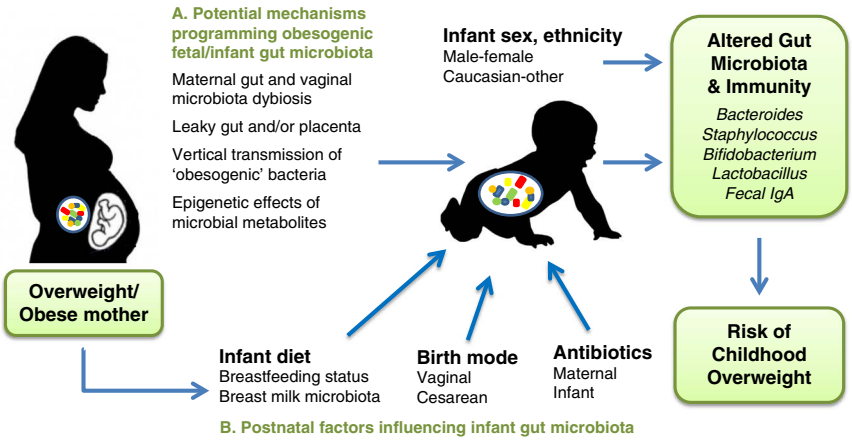

Fig. 1. Pathways of fetal programming of overweight through the microbiome. (*a*) Potential mechanisms involved in the programming of obesogenic fetal and infant gut microbiota. (*b*) Postnatal factors influenced by maternal overweight that may modify infant gut microbiota.

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was higher relative to the first trimester. Koren *et al.* also observed that the gut microbial structure of first trimester women was similar to that of healthy adults, with higher abundance of butyrate-producing Firmicutes (*Eubacterium*, *Fecalibacterium*), whereas the reduced taxonomic richness observed in the third trimester resembled that of an 'obese' microbiome. As part of their study, Koren *et al.* transferred gut microbiota of pregnant women into healthy germ free wild type mice. In doing so, they found that mice which received third trimester microbial samples had insulin resistance and greater adiposity compared with mice colonized with first trimester microbiota. Of note, streptococci in 1 month-old human fecal samples in the Koren study were equally as abundant as in fecal samples of women in their third trimester of pregnancy, but more abundant than those from first trimester pregnancies.

Gut microbial compositional changes were observed among all women in the Koren *et al.* study, but women with pre-pregnancy obesity had the lowest microbial diversity in both the first and third trimesters.[54] Using FCM-FISH and qPCR methods on the same cohort, Collado *et al.*[55] observed normal weight gain during pregnancy and pre-pregnancy BMI to be positively correlated with fecal concentrations of *Bacteroides* spp. in the third trimester ($r = 0.32$ for BMI, $P < 0.02$). Compared with normal weight women, higher levels of *Bacteroides-Prevotella*, *Bacteroides* spp. and staphylococci were found in the first and in the last trimesters of women who were overweight before pregnancy. Higher staphylococcal levels were also observed by Santacruz *et al.*[56] with overweight in the second trimester but counts of *Bacteroides* spp. were lower. Over the first 6 months of breastfeeding, Collado *et al.*[57] also reported that breast milk of overweight women had higher concentrations of staphylococci and lactobacilli, and lower counts of the bifidobacteria than of normal weight women.

The Collado group were first to demonstrate that maternal pregnancy weight could affect gut microbial composition in infants.[58] Using FISH and qPCR methods, they found that infants born to overweight mothers pre-pregnancy or to mothers with excessive weight gain during pregnancy had lower bacterial counts of genus *Bacteroides-Prevotella* ($P < 0.04$) at 1 month of age compared with infants born to mothers with normal pre-pregnancy weight or normal weight gain; pre-pregnancy weight per gram was negatively correlated with infant fecal concentrations of *Bacteroides* at this age ($r = -0.35$, $P < 0.03$). Associations with *Bacteroides* species were no longer evident in 6-month old infants in the Collado *et al.* study.[58] Instead at this age, pre-pregnancy overweight was associated with a greater likelihood of gut colonization with *C. difficile* and *Akkermansia muciniphila* in infants, and higher counts of staphylococci and *Clostridium histolyticum* but lower concentrations of bifidobacteria. Cesarean section delivery status, a more common outcome of a pregnancy complicated by overweight, was not accounted for in this study. A recent publication by Galley *et al.* reports on the longer term impact of pregnancy overweight on gut microbiota in toddlers; microbiota diversity was increased and the *Parabacteroides* spp. were more prominent in infants from overweight mothers compared with normal weight mothers, while members of the Clostridia class such as *Blautia*, were less abundant.[59] Of note, these microbial community changes were only evident among children living in high-income households and could not be attributed to the child's diet, antibiotic use or birth method.

Mechanisms for maternal gut microbiome during pregnancy and obesity risk in offspring

Despite well-known examples of bacterial migration from the maternal gastrointestinal tract to the fetus, such as neonatal infection with maternal *Listeria monocytogenes*, there is a great deal of speculation on whether prenatal overweight or other conditions can alter neonatal gut microbiota through *in utero* transmission of maternal gut microbiota. As pointed out by Solt *et al.*, some microbes colonize both the gastrointestinal and vaginal tracts.[60] For example, group B *Streptococcus*, which dominates the vaginal microbiome, is also present in the rectum. When culture-independent techniques are used, 44% of bacterial species (mostly lactobacilli) identified from either the vaginal or rectal microbiome of pregnant women can be found in both sites. Further evidence of an interaction between the intestinal and vaginal microbiomes comes from probiotic trials that report changes to vaginal microbial composition following oral supplementation with *Lactobacillus* products.[61] Finally, similar third trimester compositional changes to the gut microbiome also occur in the vaginal microbiome; namely, the bifidobacteria and lactobacilli increase in number in both microbiomes.[54,62]

The presence of identical strains in maternal gut bacteria and in newborn meconium implies that the fetus is exposed to microbes from the maternal intestinal tract.[63] As reported by Gosalbes *et al.*, microbiota in meconium can be influenced by maternal diet and even maternal smoking during pregnancy.[40] Through mechanisms not fully understood,[63] contact of maternal circulating immune cells (dendritic cells) with the paracellular space between gut epithelial cells opens up tight junctions and allows dendritic cells to directly sample microbiota from the maternal gut lumen and become internalized. Internalized intestinal bacteria can be transported to different organ systems such as the placenta, through the circulation. Changes in maternal intestinal permeability during pregnancy and delivery may play a key role in bacterial translocation.[64] Maternal obesity may also affect placental function through greater placental infiltration of macrophages and pro-inflammatory cytokines, which facilitates transfer of internalized microbes.[63]

Finally, maternal overweight can 'vertically' influence offspring weight gain through alterations of metabolites such folate and SCFAs (i.e. butyrate), which are produced by maternal gut microbiota. To indirectly test this hypothesis, Priyadarshini *et al.* undertook a study to determine the extent to which maternal serum levels of SCFAs (hypothesized to originate from microbiota fermentation in the gut) at 36–38 weeks of pregnancy were directly associated with

metabolic parameters in mothers and newborns.[65] They found that maternal serum acetate levels were associated with pregnancy weight gain; maternal serum propionate correlated negatively with newborn length and body weight. Maternal gut metabolites also have the capacity to influence epigenetic coding (DNA methylation and histone modification) of fetal intestinal cells and adipocytes to silence or express genes responsible for lipid metabolism and fat storage.[66] Adequate DNA methylation for gene expression requires folate and producers of folate, the bifidobacteria, whose levels are increased in both the intestinal and vaginal tracts in the third trimester of pregnancy.[62,66] Fecal concentrations of bifidobacteria are found to be positively correlated with plasma folate levels during pregnancy; folic acid levels are reduced in pregnant overweight women.[56] On the other hand, the gut microbiome of the first trimester is more abundant with *Roseburia* and *Eubacterium* spp., which produce butyrate, and can cause histone hyperacetylation and gene expression. In a recent study by Vidal *et al.*,[67] maternal antibiotic use during pregnancy affected PLAGL1 gene methylation status of cord blood (potentially via changes to maternal or fetal gut microbiota) to reduce birth weight.

Building toward a theory of fetal programming of infant microbiota that predict obesity risk

Synthesizing the evidence from the above gut microbiome studies in children, infants and pregnant women, and from additional probiotic trials in infants (see Table 1), we make the following arguments for a hypothesis that higher *Lactobacillus* yet lower *Bacteroides* spp. colonization in early life fecal samples (<3 months after birth) predicts risk for child overweight. This compositional structure is programmed by a pregnancy overweight, and potentially related postnatal events, including cesarean section delivery, maternal and infant antibiotic treatment, and abrogated breastfeeding:

(1) While *B. fragilis* is more common in children with a higher BMI,[22,29] in the Bervoets study, other *Bacteroides* species were less abundant and lactobacilli more abundant in overweight children.[22] In the Xu study[68] of Kazakh Chinese schoolchildren, the Bacteroidetes phylum was less plentiful in the gut of obese children, who also had evidence of insulin resistance.
(2) Colonization with *Bacteroides* species by 1 month of age has been associated with reduced growth and greater BMI

Table 1. *Studies on the association between infant and maternal microbiota and childhood obesity risk*

Reference	Study description	Findings relating to infant or maternal gut microbiota and obesity risk
Scheepers *et al.*[31]	Dutch birth cohort study (KOALA; $n = 2834$)	*Bacteroides fragilis* colonization at 1 month was associated with a higher BMI *z*-score during childhood following a low-fiber diet at age 4. Higher counts of *B. fragilis* group predicted a lower childhood BMI *z*-score in: (i) infants born to mothers adopting an anthroposophic lifestyle (organic food diet, prolonged breastfeeding) and (ii) infants in the conventional cohort only if fiber intake was low during the preschool years
White *et al.*[32]	Norwegian birth cohort study (NOMIC; $n = 218$)	Colonization with *Bacteroides* species by 1 month of age associated with reduced growth 6 months after birth
Bervoets *et al.*[22]	Belgian case–control study of overweight/obese overweight ($n = 26$) and normal weight children ($n = 27$) aged 6–16	*B. fragilis* and lactobacilli more abundant but other *Bacteroides* species less abundant in overweight children
Xu *et al.*[68]	Kazakh Chinese case–control study of overweight/obese ($n = 84$) and normal weight children ($n = 91$) aged 7–13	Lower Bacteroidetes counts in obese *v.* normal weight children
Vael *et al.*[29]	Belgian birth cohort study (Flanders region; $n = 138$)	*B. fragilis* measured at 3 and 26 weeks was positively correlated with BMI
Santacruz *et al.*[56]	Spanish case–control study of overweight ($n = 16$) and normal weight ($n = 34$) pregnant women during the second trimester	Reduced counts of *Bacteriodes* but higher amounts of lactobacilli in overweight *v.* normal weight women
Collado *et al.*[55]	Finnish case–control study of overweight ($n = 18$) and normal weight ($n = 36$) pregnant women enrolled in randomized controlled trial of a diet intervention (more fiber, fruits and vegetable and less fat) in the first and third trimesters	Higher levels of *Bacteroides* spp. during pregnancy in overweight *v.* normal weight women
Robinson and Thompson[72]	US prospective randomized trial ($n = 801$)	Infant supplementation with *Lactobacillus acidophilus* probiotic increased weight gain

BMI, body mass index.

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during childhood only with a subsequent diet low in fiber.[32] Higher counts of *Bacteroides* species in 1-month-old infants born to mothers following an anthroposophic lifestyle (organic food diet, prolonged breastfeeding) have been liked to lower BMI throughout childhood.[31]

(3) Relative to normal weight women, obese women have higher gut levels of *Bacteroides* spp. during pregnancy but lower microbial diversity in the last trimester.[54,55] Reduced counts of *Bacteroides* spp. have also been reported in pregnant overweight women.[56] Cesarean section delivery (common in overweight pregnancies) also reduces abundance of Bacteroidetes in the infant gut.[25,26]

(4) Breastfeeding, which strongly protects against child overweight,[69] is associated with lower fecal abundance of butyrate and higher abundance of *Bacteroides-Prevotella* spp. in full-term infants at 2–8 weeks of age, compared with formula feeding.[70]

(5) In preparation for birth, butyrate-producing microbes of the Firmicutes such as the *Lachnospiraceae* (*Roseburia* spp.) and *Faecalibacterium* spp., decline in the last trimester, whereas lactic acid bacteria (lactobacilli, streptococci) increase in numbers in the gut and vaginal microbiomes.[54,71] Lactobacilli have also been detected in placenta and amniotic fluid.[62] The colostrum (first milk) and second trimester fecal samples of overweight women contain higher levels of lactobacilli microbiota.[56,57]

(6) Infant supplementation with *Lactobacillus acidophilus* probiotic promotes weight gain.[72] Increases in infant fecal IgA levels have been observed after the administration of a *L. rhamnosus* probiotic mixture to infants and their mothers prenatally.[46]

The extent of gut microbial colonization with staphylococci and bifidobacteria may also play a role in overweight development. Infant colonization with bifidobacteria and staphylococci within 4 days of birth is associated with expected weight gain.[32] However, women with pre-pregnancy overweight have higher staphylococcal counts in the second and third trimester than do normal weight women[55,56]and their newborn infants are more likely to be colonized with staphylococci 6 months later.[58] Lower gut bifidobacterial counts have been observed at 3 months post birth in infants who develop overweight[28] and at 6 months of age in infants born to overweight mothers.[58] While these observations may be a function of lower breast milk intake (since breastfeeding promotes bifidobacterial growth[70,87]), the breast milk of overweight mothers at 1 and 6 months of infant age contains lower amounts of *Bifidobacterium* spp. but higher staphylococcal levels.[57] Bifidobacteria are also less enumerate in the fecal microbiota of pregnant overweight women.[56] Further study on the impact of prenatal exposures on the 'early' infant gut microbiota is needed to provide new insights on the attributes of gut microbiota profiles that initiate processes for childhood overweight and clarify how they may differ from adults. These attributes may represent whole gut microbial community profiles, generated from high-throughput gene sequencing technologies, rather than keystone species, and likely include gut immunity biomarkers, such as fecal IgA.

Sex dimorphism in associations between gut microbiota pertubations and child overweight

Increasingly we are beginning to appreciate the influence of sex of the fetus and infant in modifying microbiota associations with overweight. Several gut microbiota have been implicated in sex hormone metabolism.[73] For example, *Clostridium scindens* converts glucocorticoids to androgens. Urinary estrogens have also been found to vary according to fecal abundance of the Clostridia. A recent publication in the elderly reported lower abundance of the Bacteroidetes phylum in the gut microbiota of women.[74] In infants, most of the supporting evidence for this sex dimorphism is from antibiotic use and breastfeeding studies, although there are some indications of sex differences in gut microbial composition.

Indirect evidence from antibiotic use and breast feeding studies

Epidemiological studies of antibiotic use in infancy have provided indirect evidence for the role of the gut microbiota in the development of overweight, independent of established obesity risk factors.[75–78] The Danish National Birth Cohort and the UK Avon Longitudinal Study of Parents and Children found modest increases in risk for overweight at age 7 years and 38 months, respectively, with antibiotic exposure before 6 months of life.[75,79] A large US study of electronic health records from 65,000 children reported an association between frequent courses of antibiotic treatment within the first 2 years after birth and risk for obesity (95th percentile BMI) between age 1 and 5 years, with a stronger effect for broad spectrum antibiotics.[77]

Infant antibiotic studies are also the first to contribute evidence for sex dimorphism in obesity risk that is potentially related to the gut microbiome. Cross-sectional findings by Murphy *et al.*[78] from the International Study of Asthma and Allergies in Childhood indicated a greater association between infant antibiotic exposure and BMI in male than female children. In a prescription database-linkage study in Canadian infants, Azad *et al.*[76] reported significantly greater odds of childhood overweight at age 9 and 12 years with exposure to antibiotics during the 1st year of life for boys only. In this study, maternal overweight (measured 12 years postpartum) was crudely associated with child overweight at age 9 and 12 for both sexes, although more so in girls. Certainly, maternal eating habits are influential and more so in school-age girls than boys, but they have not accounted for associations between maternal overweight and child overweight in some studies.[80,81] Following adjustment for perinatal factors such as infant antibiotic use, tobacco smoke exposure and breastfeeding status in the Azad *et al.* study,[76] associations between maternal overweight

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(in later childhood) and child overweight were no longer evident in boys.

Evidence is emerging on sex dimorphism for other pre- and postnatal exposures, which are linked to gut microbiota. Maternal smoking during pregnancy, with reported effects on the microbiota of meconium,[40] has shown to have a greater influence on the weight of boys than girls.[82] Moreover, others have observed that exclusive breastfeeding protects against overweight development to a greater extent in boys than girls.[83] The male-specific findings for child overweight in relation to antibiotic use during infancy, and for diminished associations with maternal overweight following adjustment for antibiotic use, also posit a link between the infant gut microbiome and overweight development that is limited to boys. In addition, unpublished thesis findings from the Canadian Healthy Infant Longitudinal Development (CHILD) study indicate a higher risk for overweight (length-for-weight *z*-score >85th percentile) at age 1 among male infants following an overweight pregnancy, even when delivery mode, breastfeeding status and antibiotic use were added as covariates (adjusted OR, 3.12, 95%: 1.43–6.81).[84] No associations were found between maternal pre-pregnancy overweight and risk for overweight in female infants. Put together, these findings suggest sex dimorphism in the impact of pregnancy obesity on overweight in infants and toddlers at the time when the gut microbiome is developing.

Direct evidence from gut microbiota studies

Interestingly, no sex differences in gut microbiota composition of select taxonomic groups have been observed at 1 month of age in the KOALA cohort[25] or at multiple time points during infancy (6 days, 3 weeks, 2 months and 6 months), as assessed in the ALLADIN cohort study.[85] Neither of these cohort studies processed their infant fecal samples with gene sequencing to identify the complete microbial community. Using high-throughput gene sequencing methods, initially we also found no overall sex differences in gut microbiota diversity or composition in 3-month-old infants in the CHILD study.[86] Yet, when microbiota compositional analyses were restricted to Caucasian infants, male infants had a much lower relative abundance of *Bacteroides* species at 3 months than did female infants.

As we have summarized, members of the *Bacteroidaceae* family have been linked to weight status in adults and children, and growth in infants. According to unpublished thesis results from the CHILD study,[84] the abundance of these growth-controlling microbes in the gut of female infants at 3 months of age was unrelated to pre-pregnancy overweight. Among male infants at the same age, however, relative abundance of *Bacteroidaceae* was higher than the median in the gut microbiota of infants born to mothers who were overweight pre-pregnancy relative to women who were normal weight (OR, 3.84, 95%: 1.28–11.5). This association was independent of birth mode, breastfeeding status and antibiotic exposure. Also, as noted in this review, pre-pregnancy overweight was associated with infant risk for overweight at age 1 only among male infants in the CHILD study.

In contrast, higher gut *Bacteroides* species abundance or counts 1 month after birth has predicted lower than expected weight gain in male infants over the first 6 months of life[32] and lower BMI throughout childhood until age 10.[27] It is likely that associations with infant growth are sensitive to critical windows in gut microbiota maturation and infant diet. In both of the aforementioned studies, most infants were breastfed for an extended period and no associations were found between *Bacteroides* concentrations and infant growth beyond 1 month of age.[32] Also in the Scheepers *et al*. study,[31] *Bacteroides* counts were positively correlated with childhood BMI only among infants subsequently fed a low-fiber diet. Among (predominantly formula-fed) infants in the Vael *et al*. study,[29] positive correlations were found between fecal concentrations of *Bacteroides* and BMI between 3 weeks and 6 months of birth, but not at 1 year of age. Finally, it is quite plausible that initial gut microbiota colonization is a critical window that determines infant growth trajectories. In the White *et al*. study,[32] colonization with *Lachnospiraceae* (includes *Roseburia* spp.) and *Bifidobacterium longum* 4 days after birth was associated with reduced growth during the next 6 months in male but not female infants. Findings from Vidal *et al*.[67] on maternal antibiotic treatment during pregnancy suggest the possibility that gut microbiota-mediated changes in infant weight can be initiated *in utero*, preferentially affecting male newborns.

Influence of ethnicity and geography in gut microbiota programming of child overweight

Stratification by ethnicity in antibiotic use or microbiotia studies has uncovered sex differences among Caucasian infants only, for example, sex differences in birth weight according to prenatal antibiotic exposure[67] and in gut microbiota composition. For certain, both geography and ethnicity are a source of variation in gut microbiota composition.[87–89] A recent meta-analysis of microbiota by Lozupone *et al*.[90] documented a clear separation in the gut microbiota profiles of adults and children of industrialized cultures (USA, Italy) from that of the developing world (Burkina Faso, Malawi, Venezuela). However, ethnic and geographic area variation is presently understudied in relation to their influence on infant gut microbiota composition, and childhood obesity or other health outcomes. Compared with school children in the United States, the gut microbiota of Bangladeshi children was found to harbor greater bacterial diversity and be enriched with *Prevotella* but depleted in *Bacteroides* spp.[91] Of note, the majority of the children in the US sample of this study were of Chinese ethnicity. It is likely that many ethnic and geographic differences in gut microbiota structure arise during infancy. In the Yatsunenko *et al*.[89] study, breastfed infants in the United States had a higher abundance of the Bacteroidiales order in their gut and were less often colonized with lactobacilli than infants in Africa or South America.

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Concluding remarks

Maternal and childhood obesity are concerning public health issues facing the world today. Evidence is accumulating on the importance of the prenatal period in determining risk for obesity in childhood and for its role in the establishment and development of the gut microbiome. Newer generation gene sequencing methods have advanced our knowledge on the disruptive effect of birth interventions and postnatal exposures on infant gut microbiota development but little is known about the impact of prenatal exposures such as maternal overweight, and how they may affect infant overweight risk. This gap in knowledge motivated us to survey human studies in this field, with the goal of informing future research on prenatal exposures that affect gut microbiota maturation and predict childhood overweight. Only with additional epidemiologic evidence and mechanistic insight from human and animal model studies can microbiome research inform prenatal care, and ultimately reduce the risk and burden of childhood overweight and associated chronic diseases in our society.

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Conflicts of Interest

None.

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