

REVIEW

Impact of maternal nutrition in pregnancy and lactation on offspring gut microbial composition and function

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ABSTRACT

Evidence supporting the Developmental Origins of Health and Disease Hypothesis indicates that maternal nutrition in pregnancy has a significant impact on offspring disease risk later in life, likely by modulating developmental processes *in utero*. Gut microbiota have recently been explored as a potential mediating factor, as dietary components strongly influence microbiota abundance, function and its impact on host physiology. A growing body of evidence has additionally indicated that the intrauterine environment is not sterile as once presumed, indicating that maternal-fetal transmission of microbiota may occur during pregnancy. In this article, we will review the body of literature that supports this emerging hypothesis, as well as highlight the work in relevant animal models demonstrating associations with maternal gestational nutrition and the offspring gut microbiome that may influence offspring physiology and susceptibility to disease.

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Introduction

The importance of nutrition in pregnancy to neonatal health is underscored by the decades of work with the Developmental Origins of Health and Disease (DOHaD) Hypothesis, which proposes that adverse *in utero* conditions can influence developmental pathways in early life that results in long-term changes to offspring disease susceptibility.¹⁻⁷ A key aspect of this work demonstrates that low birthweight is associated with increased incidence of cardiovascular disease in adulthood,^{2,8} leading to ‘Barker’s Hypothesis’, which argued for fetal programming of adult disease.² Despite substantial epidemiologic evidence that supports this hypothesis, the underlying biological mechanisms are not entirely understood. Changes to the epigenetic regulation of gene expression induced by altered maternal diet has been shown in both murine and monkey models,⁹⁻¹⁷ but given the complexity of obesity, metabolic disease and other developmental disorders, it is unlikely to be the only mediating factor.

Recent work on the human microbiome indicates that gut microbiota may additionally explain the

observations put forth by the DOHaD Hypothesis. The importance of microbiota to human health and disease has come into the scientific spotlight within the last decade,¹⁸⁻²² and major questions surround how and when we acquire our microbiota.²³⁻²⁷ It is widely recognized that microbial transmission of bacteria from mother to offspring is essential for the establishment and development of a healthy nascent microbiome, which may impact infant growth,²⁸⁻³⁰ immune system maturation³¹⁻³⁵ and even neurodevelopment.^{36,37} Interestingly, emerging evidence has additionally indicated that transmission of microbiota from mother to offspring may occur before delivery, which has since reemphasized the importance of the pregnancy period to the development of the neonatal microbiome. Because diet has been shown to substantially affect the type and abundance of microbiota within the human gastrointestinal tract,³⁸⁻⁴³ we and others have begun to explore how maternal diet during pregnancy may impact the early establishment of the neonatal microbiome. Given the multifaceted nature of this topic, in this article we will briefly review

how pregnancy impacts the maternal microbiota and the ways in which offspring may inherit microbiota from their mothers, both during pregnancy and in immediate postnatal life. Finally, we will examine how maternal diet during pregnancy and lactation may impact these processes, and to what extent this may influence offspring health and disease later in life.

Influence of pregnancy on the maternal microbiome

While the oral cavity, urogenital tract, intestinal tract, and skin have been thoroughly characterized in the non-pregnant population,^{18,19,44} there have been relatively few studies examining alterations in the microbiome by virtue of pregnancy. However, during pregnancy and lactation, the physiology of nearly every organ system of the mother undergoes profound changes to support the rapid growth and development of her offspring (For a review see ref⁴⁵). A recent study by DiGiulio and colleagues examined the vaginal cavity, oral cavity, and stool by virtue of gestational age and concluded that there was minimal remodeling of an individual's microbiome associated with pregnancy.⁴⁶ However, this is in contrast to prior studies examining the microbiome during pregnancy. Aagaard et al. was one of the first to demonstrate that the vaginal microbiome was altered by virtue of pregnancy. In this cross-sectional study, the microbiota of the vagina from 24 gravidae and 60 non-gravidae was examined, and it was demonstrated that the vaginal microbiome was altered by virtue of pregnancy.⁴⁷ These alterations were demonstrated by a decrease in α diversity and an increase in the abundance of *Lactobacillus* species that was associated with pregnancy.⁴⁷ Subsequently, 2 independent studies further examined the vaginal microbiome longitudinally during pregnancy through self-collection methods.^{48,49} These studies were in agreement with Aagaard et al. and demonstrated that α diversity decreased and *Lactobacillus* species increased with increasing gestational age.^{48,49} Altogether, alterations in vaginal microbiota are associated with pregnancy with an increased abundance in *Lactobacillus* species. This may be a mechanism to increase lactic acid production in the vaginal cavity to protect the fetus from infection.⁵⁰

In addition to studies examining the microbiome of the vaginal cavity during pregnancy, there are a handful of studies examining the gut microbiome during

pregnancy. These studies have resulted in hypothesizing that commensal bacteria within the gastrointestinal tract (GIT) of the mother play 2 major roles during pregnancy: supporting the increased energy demands required for rapid development and growth of the offspring and providing the foundation for a nascent microbial community within the offspring. The latter hypothesis developed after the pivotal study by Koren et al. examining the gut microbiome during pregnancy of 91 Finnish gravidae.⁵¹ This study examined the gut microbiome of gravidae during the first and third trimesters and found that significant changes in the microbiome occurred between these gestational ages. With an increase in gestational age, increases in the abundance of Proteobacteria and Actinobacteria occurred in the third trimester.⁵¹ Intriguingly, gestational age (first versus third trimester) was associated with significant increases in leptin, cholesterol, and markers of insulin resistance in plasma along with increases of pro-inflammatory cytokines (i.e. IFN γ , IL-2, IL-6, and TNF α) in the stool. Furthermore, these increases occurred despite no significant changes in dietary intake of fat, carbohydrates, protein, etc.⁵¹ While no significant metabolic changes occurred by virtue of gestational age as measured by metagenomic sequencing, germ-free mice receiving stool from gravidae during the third trimester had increases in adiposity, blood glucose, and inflammatory cytokines in comparison to germ-free mice receiving stool from gravidae during the first trimester.⁵¹ Altogether, this data demonstrates that the gut microbiome is altered by virtue of pregnancy. However, the recent study by DiGiulio et al. found no significant alterations in the gut microbiome associated with gestational age by β nor α diversity.⁴⁶ The discrepancy between the 2 studies may be due to study design (cross-sectional vs. longitudinal) and the subject participants (Finnish versus American); however, this discrepancy highlights the need for further examination of the gut microbiome, particularly with regard to diet. A prior study by Collado and colleagues has demonstrated alterations in the gut microbiome during pregnancy by virtue of obesity and gestational weight gain. In this study, fluorescence *in situ* hybridization (FISH) and quantitative real-time PCR (qPCR) were utilized to examine the gut microbiome of pregnant women that were normal weight or overweight and that had normal or excessive gestational weight gain.⁵² An increase in the abundance of *Staphylococcus*

aureus was found to be associated with pregnant subjects that were overweight or had excess gestational weight gain.⁵² Thus, with the onset of Next-Generation (Next-Gen) sequencing, in-depth examination of the microbiome, pregnancy, and diet is now possible and further study is needed to understand how interactions between diet, pregnancy, and the microbiome impact maternal and fetal health.

Impact of maternal nutrition in pregnancy on offspring gut microbiota

It has been historically thought that the fetus and intrauterine environment is sterile, with the newborn's first contact with microbiota coming at the time of parturition. However, observations of healthy pregnancies and studies within relevant animal models have indicated that the fetus may be first exposed to bacteria during gestation. Both culture and PCR based techniques have positively identified bacteria in the fetal membranes,^{53,54} cord blood⁵⁵ and amniotic fluid⁵⁶⁻⁵⁸ of healthy, term pregnancies, suggesting that microbiota can inhabit the *in utero* environment without overtly affecting the pregnancy or the health of the infant. Additionally, the use of Next-Gen sequencing technologies revealed the diversity of the low biomass microbial community of the placenta parenchyma,^{59,60} which was also historically considered a sterile tissue in the absence of disease. Across the 320 placentae examined in the Aagaard study, the most common bacterial species identified were Proteobacteria, such as *Escherchia coli*, and other microbiota common to the oral cavity, such as *Fusobacterium* and *Streptococcus* species.⁶⁰ This work has since galvanized efforts to reconsider our fundamental assumptions about when and whence we first being to acquire our microbes in early life. Observations of mother-neonatal pairs by Dong et al. and Collado *et. al.* demonstrated that the microbiota found within the placenta share significant similarity to that of the neonate's meconium,^{57,61} as well as the amniotic fluid,⁵⁷ indicating that microbiota may be transferred across the placenta at the maternal-fetal interface into the intrauterine space.

Evidence for such a mechanism has been suggested by previous work in animal models. In an early study, Jimenez and colleagues orally administered genetically labeled *Enterococcus fecium* to pregnant mice and sterilely delivered their pups one

day ahead of anticipated delivery.⁶² Interestingly, they could culture and identify the labeled bacterium from the fetal intestine, indicating that microbiota can be transferred from mother to offspring even before delivery occurs.⁶² However, the precise route of transmission was not examined in this study and to date remains unclear. Work by Han et al. and Fardini et al. has put forth a hematogenous model of placental colonization that potentially explains these observations.^{63,64} In the former study, *Fusobacterium nucleatum* was given to pregnant mice intravenously during late gestation (embryonic day 16-17). While peripheral organs cleared *F. nucleatum* within 24 hours, this bacterial species persisted in the placenta and could be detected in the amniotic fluid and fetus at 72 hours post-infection.⁶⁴ In the latter study, the authors intravenously administered commensal bacteria typical of the human oral cavity to pregnant mice late in gestation, and found that they could selectively detect many of these administered microbiota in the placental tissues by PCR.⁶³ However, the fetal tissues were not specifically examined in this study and thus a hematogenous route of placental and subsequently fetal colonization remains speculative without more definitive evidence. Ascending colonization from the vagina has been alternatively hypothesized as a potential origin largely in part due to its anatomical proximity to the intrauterine environment and its association with preterm birth.^{65,66} However, as aforementioned, the vagina tends to predominately be populated by *Lactobacillus* species before pregnancy, and tends to be further enriched for *Lactobacilli* as the pregnancy progresses.⁴⁶⁻⁴⁸ Although *Lactobacillus* species have been detected in the placental membranes in healthy, term pregnancies by Next-Gen sequencing,⁶⁷ the overall diversity of commensal species found within the placental parenchyma, amniotic fluid and neonatal meconium suggest that the vaginal microbiome is unlikely to be the only origin for the full gamut of microbial species found within the intrauterine space. Nevertheless, well-designed animal studies are required to further refine these observations and better define a model of microbial transmission during this period.

If the neonatal gut microbiome is established during pregnancy, then it stands to reason that maternal exposures during pregnancy, such as maternal

nutrition or inflammation, could impact microbial transmission during this period, leading to long-term consequences to how the offspring gut microbiome develops in postnatal life. Recent studies have highlighted that maternal influences, such as excess gestational weight gain and inflammation, are associated with alterations in the placental microbiome.^{67,68} Subjects with excess gestational weight gain that subsequently deliver preterm were found to have a decrease in Proteobacteria and an increase in Firmicutes.⁶⁸ Furthermore, inferred metagenomic pathway analysis indicated that butyrate metabolism was decreased in preterm subjects with excess gestational weight gain.⁶⁸ As butyrate has been shown to decrease inflammation in the gut,^{69,70} preterm subjects with excess gestational weight gain may have increased inflammation in the placenta. Examination of alteration in the placental membrane microbiome in association with inflammation was recently examined by Prince and colleagues. In this study, placental inflammation was examined in the context of histological chorioamnionitis. The authors confirmed that the placental membrane microbiome was altered by virtue of gestational age as was seen with the placental parenchyma microbiome.⁶⁷ Additionally, the placental membrane microbiome could be demarcated by virtue of histological chorioamnionitis.⁶⁷ Furthermore, work in a non-human primate model has demonstrated that placental inflammation is increased with a maternal high fat diet.^{71,72} Intriguingly, work in this same model has provided early evidence that the offspring microbiome is altered in association with maternal diet.³⁹ In this study, dams were either provided a high-fat diet or a control diet prior and during pregnancy and lactation. As with humans, a high-fat diet caused significant weight gain and induced corresponding shifts in the non-pregnant gut microbiome.^{38,39} To isolate the effects of maternal diet, the offspring of both high-fat and control dams were weaned onto a control diet at 6-7 months of age. Interestingly, at 1-year of age, the offspring gut microbiota could be discriminated based on whether their mothers consumed a high-fat or control diet, despite the offspring consuming a control diet for several months.³⁹ Specifically, a high-fat diet appeared to persistently diminish the relative abundance of commensal *Campylobacter* species in the offspring gut,³⁹ indicating that maternal diet may play a significant role in shaping the transmission of commensal

microbiota that can persist beyond infancy and may extend into adulthood. We've recently extended these observations to humans, similarly demonstrating in a large population-based prospective cohort that independent of breastfeeding status, mode of delivery and maternal obesity, the early infant gut microbiota at delivery and within the first 6 weeks of life differs by virtue of a maternal high-fat diet (>40%) within the 3rd trimester of pregnancy.⁷³ However, in this study, *Bacteroides* species were depleted in the stool of infants exposed to a maternal high-fat gestational diet,⁷³ which is notable given the critical role that *Bacteroides* species appear to have in modulating host metabolism and immune system development within the gut mucosa during early development.⁷⁴ Altogether, studies highlighting inflammatory modulations of the placental microbiome combined with associations of maternal diet with the offspring gut microbiome suggest that seeding of the microbiome may occur *in utero* or very early in life.

Impact on offspring health and disease

In keeping with the DOHaD Hypothesis, parallel work in mouse models has demonstrated that early aberrations to the microbiota of the early neonatal gut associated with a maternal high-fat gestational diet can lead to pronounced differences in offspring physiology and behavior later in life. This includes the development of obesity, which has previously been attributed to gut microbiota in adults.^{22,75-77} Gut microbiota metabolize many indigestible dietary components into useful compounds that impact host cellular processes or that can be subsequently utilized for energy.^{74,78} Interestingly, in the absence of microbiota, mice raised in completely sterile conditions (germ-free mice), weigh less than their conventional counterparts, demonstrate reduced adiposity, and are resistant to high-fat diet induced obesity,^{79,80} suggesting that gut microbiota play a significant role in mediating the impact of diet on obesity pathophysiology. Limited evidence to date has similarly indicated that maternal gestational diet may influence offspring adiposity in early life by altering offspring gut microbiota. Independent studies have implicated certain bacterial species, including *E. coli*, as a major modifying factor of this phenotype in the mouse,⁸¹ though it is uncertain if *E. coli* has a similar impact on infant adiposity and

growth trajectories in humans. Intriguingly, mitigating the effects of a high-fat diet on the maternal gut microbiota with a prebiotic supplement appears to attenuate the impact of maternal diet on the offspring's propensity for adiposity,⁸² indicating that additional dietary manipulations could be useful to offset the long standing impact of a sustained high-fat diet. However, the development of obesity is an incredibly complex pathophysiological process that may be first programmed in fetal life, but is likely sustained in postnatal life by continued environmental exposure to high-density dietary intake or aberrant microbiota.

Altered gut microbiota in early life associated maternal gestational diet may have additional consequences to the programming of the offspring immune system in early life. Through decades of work on germ-free animals, it is known that commensal microbiota are essential for the proper patterning of both innate and adaptive immunity, which is unsurprisingly dependent on specific host interactions with specific microbial species (For a review see refs ^{32,83,84}). Reintroduction of microbiota in the postnatal period can partially correct many of these immune defects, though even a brief germ-free period can induce immunological changes that persist into adulthood.³⁴ Recent work by Gomez de Augero et al. has further highlighted the interplay between maternal microbiota and the offspring immune system by utilizing a model system where murine dams are transiently colonized with genetically engineered *Escherichia coli* during pregnancy.⁸⁵ The authors found that the frequency of immune cells, such as innate lymphoid and mononuclear cells, was increased when dams were colonized with bacteria during gestation. Furthermore, gene expression of intestinal tissue was altered by transient colonization of *E. coli* during pregnancy, and microbial molecules could be found in offspring tissues postnatally as detected by radio-labeled bacteria that was given to dams during pregnancy. With this recent insight, it is plausible that dysbiotic alterations in the maternal gut microbiota as a result of dietary influences may have long-term effects on the development of inflammatory related disorders later in life. Indeed, Myles and colleagues demonstrated in a murine model that offspring exposed to a high-fat diet during gestation had a decrease in T regulatory cells with a corresponding increase in

autoimmune disorders, such as experimental autoimmune encephalitis. Conversely, offspring exposed to a high fat diet during gestation were also more susceptible to pathogenic bacterial infections.⁸⁶ Furthermore, feeding pregnant mice a high-fiber diet lead to changes in the maternal gut microbiota that were associated with increased short chain fatty acid production and suppression of allergic airway disease in the offspring.⁸⁷ With the uncertainty surrounding the time frame of maternal dietary exposure during pregnancy that is associated with alterations in the offspring immune phenotype, it is important to be mindful of maternal dietary components when examining host-microbial modulations of the offspring immune system in early life.

In addition to obesity and immunity, recent data suggests that maternal diet may have an impact on offspring behavior by modulating gut microbiota. Bidirectional communication between the brain and the enteric nervous system has long been recognized, but only within the last few years has the impact of gut microbiota been explored in greater detail (For a review see ref ³⁶). By producing neurotransmitters in the gut, such as serotonin or GABA, gut microbiota are hypothesized to contribute to a number of neurological and behavioral disorders, including anxiety, depression and autism, by activating or depressing neural pathways in the enteric and central nervous system.³⁶ Recent work in the mouse by Buffington et al. has indicated that maternal gestational diet may modify offspring behavior by altering offspring gut microbiota in early life.⁸⁸ In this study, offspring whose mothers consumed a high-fat diet in pregnancy exhibited profound social deficits associated with significant changes to oxytocin levels in the brain and specific alterations of the offspring gut microbiota.⁸⁸ Intriguingly, postnatal reintroduction of *Lactobacillus reuteri*, which was depleted as a result of a maternal high-fat diet, to the affected offspring was found to ameliorate the deficient social behavior and enhance oxytocin levels in the brain, indicating a causal linkage between maternal diet, gut microbiota, neurologic development.⁸⁸ Thus, future studies examining the impact of maternal gestational diet on offspring gut microbiota will likely continue to refine our understanding of which microbiota are important to neurologic development, how these microbiota are capable of modulating the gut-brain axis, and when these interactions are required.

Impact of maternal nutrition during breastfeeding

The impact of maternal nutrition on the offspring gut microbiome and associated health outcomes likely continues beyond pregnancy into the postnatal period through breastfeeding. Indeed, many studies, including several of the ones aforementioned, cannot or do not separate dietary influences during the gestation and lactation; thus the contribution of the postnatal period to long-term health outcomes in offspring should be considered when interpreting these studies. Cross-fostering studies in mice have highlighted the importance of maternal diet during lactation to offspring health. Pups cross-fostered to dams fed a high fat diet during lactation develop symptoms of metabolic disease including hypertension,⁸⁹ diet-induced obesity,⁹⁰ and insulin resistance.⁹¹ While the mechanisms underlying these findings are poorly understood, dietary-mediated changes to human milk composition likely represent a major contributing factor. Given the significant association between microbiome composition and metabolic disease outcomes,⁹² maternal dietary influence on offspring disease outcomes may be partially explained by the dietary modulation of human milk components impacting the offspring gut microbiome.

Human milk is a highly complex nutrient source that has multiple nutritive and bioactive components with potential to impact the developing offspring microbiome. Once thought to be sterile, it is now well-established that human milk contains a distinct microbiome consisting of diverse species.^{93,94} These bacteria seed the gastrointestinal tract of the breastfeeding infant, likely contributing to the significant shifts in microbiome composition associated with breastfeeding.⁹⁵⁻⁹⁷ Intriguingly, in addition to skin-associated (*Staphylococcus*) and oral-associated (*Streptococcus*) taxa, the milk microbiome includes anaerobic bacteria most commonly associated with the gut such as *Bifidobacteria* and *Enterococcus*.⁹⁴ The origin of these bacteria has yet to be fully elucidated, but evidence suggests that these bacteria may be translocated from the maternal gut via enteromammary trafficking, a pathway in which bacteria in the gut lumen are engulfed by leukocytes through the process of antigen sampling and translocated intracellularly to the mammary glands via systemic circulation (For a review see ref⁹⁸). In support of this hypothesized pathway, a

study of mothers given oral *Lactobacillus* probiotics for the treatment of mastitis showed that the *Lactobacillus* strains were detected in the breast milk of 6 out of 10 mothers after oral probiotic administration.⁹⁹ Studies of mother-infant pairs have shown that multiple species of bacteria, including gut-associated anaerobes, are common among maternal stool, breast milk, and infant stool, and that the number of shared species between maternal and infant stool significantly increases with time.^{100,101} As profiling by sequencing does not necessarily indicate that transferred bacteria are viable, one study demonstrated that a viable strain of *Bifidobacterium breve* was shared among maternal stool, breast milk, and infant stool from a mother-infant pair.¹⁰⁰ Whatever their origin may be, it is tempting to speculate that these gut-associated bacteria play a key role in establishing the gut microbiome of breastfeeding infants. Additionally, as diet is a strong driver of the adult gut microbiome,³⁸ enteromammary trafficking may represent a mechanism by which dietary-mediated shifts in enteric bacteria are transferred from mother to infant postnatally. However, the effect of maternal diet on the milk microbiome has not been explored, and represent a vitally important focus of future research efforts.

Human milk contains many other components with the potential to transmit maternal dietary influence to the offspring microbiome, including macronutrients, human milk oligosaccharides, and immune factors such as maternal immunoglobulins (i.e., IgA). High fat maternal diet significantly affects fat and energy content in human milk, which may in turn affect proliferation of bacteria in the infant gut.^{102,103} Human milk contains a relatively high abundance of undigestible oligosaccharides (human milk oligosaccharides, HMOs) that favor proliferation of specific bacteria in the infant gut such as *Bifidobacterium* spp.¹⁰⁴ The HMO profile of breast milk varies substantially among women, but the effect of maternal diet on HMO composition has not been well characterized.¹⁰⁴ Finally, human milk contains a high abundance of IgA that protects nursing infants from infections by providing passive immunity. While it is presumed that IgA preferentially targets pathogens, its role in molding the infant gut microbiome has not been well explored. Intriguingly, diet has been shown to modulate IgA production in intestinal and extra-intestinal mucosal tissues as well as alter IgA-coating of bacteria in the gut microbiome.¹⁰⁵ Further studies

are needed to characterize how diet affects maternal IgA content in human milk and the role of maternal IgA in shaping the offspring gut microbiome.

Conclusions

In conclusion, maternal nutrition during pregnancy and lactation appears to have a substantial impact on offspring physiology and behavior by altering the abundance of prevalence of offspring gut microbiota in early life. However, the mechanisms through which this occurs remain unclear. Maternal diet in pregnancy and lactation likely impacts the abundance and prevalence of microbiota found within her microbiome, thus changing the pool of bacteria capable of being transferred to the offspring during gestation and early life. Microbial transmission across the maternal-fetal interface in the placenta has been put forth as a potential mechanism, but additional studies are required to fully validate this model. Additionally, it is hypothesized that maternal gut microbiota continue to be transferred to the infant gut in postnatal life through breastfeeding via an enteromammary pathway, but further work is similarly needed to refine these observations. It is also important to consider the independent effects of dietary components on host physiology. For instance, a maternal high-fat diet alters epigenetic programming in the infant that may induce inflammation. Therefore, the effects of maternal diet on offspring health and disease may be exerted through a combination of mechanisms, some of which may be transient and reversible (gut microbiota, inflammation), and others that may be more persistent (epigenetics). Nevertheless, incorporating gut microbiota into the DOHAD hypothesis will enable investigators to better understand the impact of maternal nutrition on offspring health and disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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