Review

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Katie M. Strobel *, Sandra Juul *, D. Taylor Hendrixson

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Review

Malnutrition and the Microbiome across the Four Trimesters of Pregnancy and the Post-Partum Period

Katie M Strobel *,†, Sandra Juul * and D Taylor Hendrixson †

Department of Pediatrics, University of Washington School of Medicine, 1959 NE Pacific St, Seattle 98195, WA, USA

* Correspondence: kmstrob@uw.edu

† These authors contributed equally to this work.

Abstract: Appropriate nutrition during pregnancy and the post-partum period is vital to both the parent and their offspring. Both under- and over-nourished status may have important microbial implications on the parental and infant gut microbiomes. Alterations to the microbiome can have implications for a person's risk of obesity and metabolic diseases. In this review, we examine alterations in the parental gut, vaginal, placental, and milk microbiomes in the context of pre-pregnancy BMI, gestational weight gain, body composition, gestational diabetes, and parental diet. We also investigate how the infant gut microbiome may be altered by these different parameters. Many of the microbial changes seen in under- and over-nourished states in birthing parents may result in long-term implications to the health of offspring. Differences in diet appear to be a major driver of the parental, and subsequently milk and offspring microbiomes. Further prospective longitudinal cohort studies are needed examining nutrition and the microbiome to better understand its implications. Additionally, trials involving dietary interventions in child-bearing age adults should be explored to improve the parent and child's risks for metabolic diseases.

Keywords: parental nutrition; obesity; pregnancy; microbiome

INTRODUCTION

The pregnancy and post-partum periods are times of significant metabolic and microbial change for the birthing parent and their offspring. There are increased energy requirements to support the growth of the uterus, breast tissue, placenta, and fetal tissues.[1] The gut microbiome facilitates nutrient absorption, gut defense barrier, and immune development. There is an increase during pregnancy in gut *Akkermansia*, *Bifidobacterium*, and Firmicutes bacteria, likely to facilitate energy storage.[2] There is also an increased abundance of gut bacteria Proteobacteria and Actinobacteria which are believed to protect against inflammation.[2] The nutritional status of the pregnant person is important in regulating these microbial shifts necessary in pregnancy and the post-partum period.

Both excessive and inadequate nutrition can have remarkable consequences to the parent during pregnancy (**Table 1**). Obesity, excessive gestational weight gain, gestational diabetes, and hypertension have been associated with increased future risk for obesity, cardiovascular disease, and type 2 diabetes.[1] In contrast, inadequate nutrition in pregnancy has been associated with increased risk of life-threatening hemorrhage, obstructed labor, sepsis, and all-cause mortality. [3–5]

Table 1. Maternal intestinal microbiome changes due to various anthropometric and nutritional states. References are listed in brackets.

Maternal Factor	Diversity	Increased Abundance	Decreased Abundance
Elevated pre-pregnancy BMI	Potentially decreased diversity [13]	Firmicutes [14], *Bacteroides* [16], Clostridium[16], *S. aureus* [16] *Biolphila* [17], *Roseburia* [17], *Dialster* [17],	Proteobacteria [14], *Phascolarctobacterium* [17]

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Underweight	Potentially decreased [35,43,46]	*Acidaminococcus* [47]	Firmicutes [44], Bacteroidetes [44]
Excessive Gestational Weight Gain		*Prevotella* [17], *Dialister* [17], Firmicutes [49], Bacteroidetes [49]	*Bifidobacterium* [16]
Gestational Diabetes		Ruminococcaceae family [66], *Faecalibacterium* [67], *Eubacterium* [67], *Streptococcus* [67], Enterobacteriaceae family [67], *Bacteroides* [68], *Ruminococcus* [16,17], *Paraprevotella* [17]	
Fat Intake	Increased Simpson diversity [17]		Bacteroidetes, Firmicutes [74]
Vegetable Intake		*Roseburia* [79], *Lachnospiraceae* [79]	*Collinsella* [79], *Holdemania, Eubacterium* [79]
Animal Protein Intake	Increased Shannon diversity [17]	*Collinsella* [17]	
Carbohydrate Intake		Bacteroidetes [74]	

Excessive and inadequate nutrition can also have consequences to the offspring (**Table 2**). The effects of parental nutritional status on infant outcomes may be mediated through several pathways including dietary intake, milk composition, and parental microbiome (**Figure 1**). Infants of parents with gestational diabetes and/or pre-pregnancy obesity have increased risk of increased fetal growth, large for gestational age status at birth, and later metabolic syndrome.[6] Inadequate nutrition during pregnancy alters placental histomorphology and function [7] and can lead to epigenetic changes regarding nutrient utilization, as well as a higher risk of fetal growth restriction, small for gestational age status, and later metabolic syndrome.[7] Additionally, human milk oligosaccharides (HMOs) can be altered by nutritional status[8,9] and are pre-biotics for milk and gut bacteria. Thus, HMOs may alter the milk microbiome and shape the infant gut microbiome.[10–12]

Table 2. Infant gut microbiome changes due to various anthropometric and nutritional states. References are listed in brackets.

Maternal Factor	Infant Gut Microbiome Diversity	Infant Gut Microbiome Increased Abundance	Infant Gut Microbiome Decreased Abundance	Infant Gut Microbiome Functional Roles
Elevated Pre-pregnancy BMI	Increased [34]	Proteobacteria [14] Vaginal delivery infants [35]: *Bacteroides fragilis, Escherichia coli, Veillonella dispar, Staphylococcus, Enterococcus*	Firmicutes [14]	Decreased butyrate production [36]
Gestational Weight Gain	Increased [34]		*Akkermansia* [34]	Enrichment of glucose and glycogen degradation pathways, increased phenylalanine, cysteine/serine,

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				folate, thiamin, biotin, and pyridoxine synthesis pathways [56]
Gestational Diabetes	Decreased [72]	*Clostridium* [73], *Veillonella* [73], Firmicutes [72], *Streptooccus* [66]	Proteobacteria [72], *Lactobacillus* [66,75], *Flavonifractor* [75], *Erysipelotrichaceae* [75], *Gammoproteobacteria* [75], *Bacteroides* [66]	
Maternal Fat Intake		Firmicutes [76]	Proteobacteria [76], *Bacteroides* [78]	
Maternal Fruit and Vegetable Intake		*Lactobacillus* [79], Propionibacteriales [86], Priopionibacteriaceae [86], *Cutibacterium* [86], Tannerellaceae [86], *Parabacteroides* [86], *Lactococcus* [86]	*Coprococcus* [76], *Blautia* [76], *Roseburia* [76], *Rumiococcaceae* [76], *Lachnospiracea* [76]	
Maternal Animal Protein		*Veillonella* [76], *Escherichia/Shigella* [76], *Klebsiella* [76], and *Clostridium* [76]		

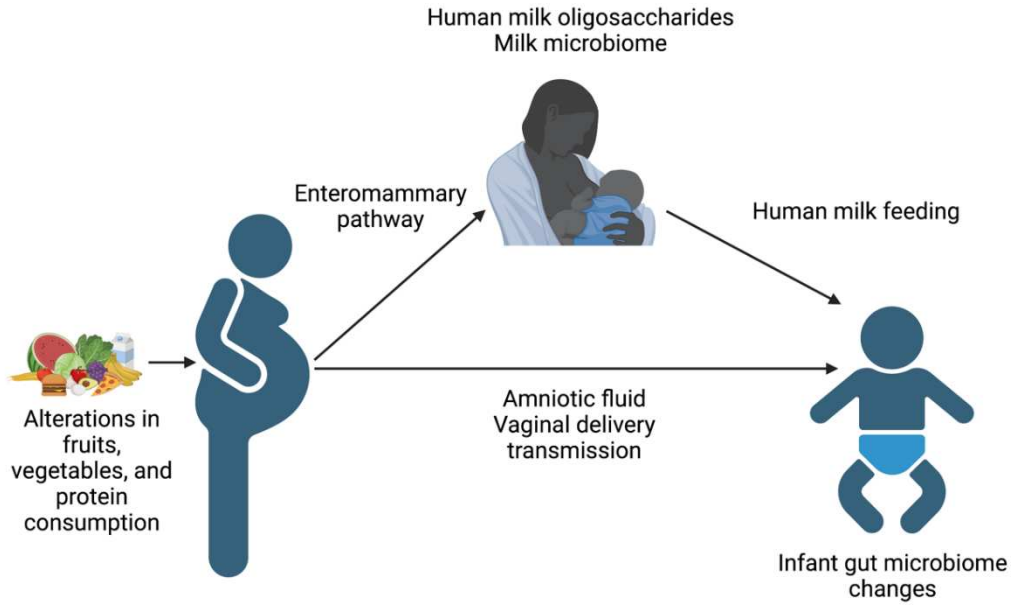

Figure 1. Schematic of the pathways maternal nutrition influences the infant gut microbiome. Figure created with Biorender.com.

Alterations in the microbiome have been associated with inappropriate nutrition in pregnancy. Both obesity and undernutrition in pregnancy have been associated with decreased gut microbial diversity and shifts in microbial abundance.[13] However, changes in diet can modify the microbiome of the mother and offspring. In this review, we will explore the impact of different markers of metabolic health and diet on the parental and infant microbiome in pregnancy and postpartum period.

PRE-PREGNANCY BMI AND ITS ROLE IN MICROBIAL CHANGES

OVERWEIGHT/OBESITY

Increases in pre-pregnancy body mass index (BMI) have been associated with alterations to the parental gut microbiome. In the first trimester, pregnant parents who were obese prior to pregnancy were found to have a higher relative abundance of Firmicutes and a lower relative abundance of Proteobacteria compared to their normal body weight counterparts.[14] A high abundance of Firmicutes has been found in multiple studies of adults with obesity, with evidence suggesting that Firmicutes increase the efficiency of energy extraction and promote the absorption of calories.[15] In the third trimester, overweight pregnant parents compared to normal BMI parents were found to have increased *Bacteroides*,*[16] Clostridium*,[16] *Biolphila*,[17] *Roseburia*,[17] *Dialster*,[17] and *S. aureus* [16] and decreased *Phascolarctobacterium* compared to normal BMI parents.[17] *S. aureus* have been found in the presence of intestinal inflammation secondary to adipocyte hyperplasia [15]. However, these changes are inconsistent across studies- one study found no changes in genus-level composition between those with an elevated BMI compared to those with a normal BMI.[18] Similarly, some studies have found decreased diversity with obesity [13] while others have not.[17]

Obesity also has been associated with alterations to the parental vaginal and placental microbiomes. Alterations in the vaginal and placental microbiome have been associated with preterm birth.[19] Women with normal weight have with increased diversity in the vaginal microbiome in the introitus and post-fornix compared to those with obesity.[20] Another study in Caucasian parents found pre-pregnancy BMI was associated with an elevated Nugent score, a score concerning for vaginal dysbiosis[21]. The role of obesity versus gestational weight gain on the placental microbiome has been controversial. Parental obesity is believed to contribute to placental dysfunction. In a pig model of obesity in pregnancy, the obese group had increased oxidative damage with increased reactive oxygen species protein[22]. These reactive oxidative species and interleukins were correlated with relative abundance of *Christensenellacaea_R-7* and decreased *norank_f_Bacteroidales_S24-7_group*, members of Firmicutes phyla. These findings suggest that placental inflammation may be secondary to microbial changes and could contribute to risk for preterm birth. However, when examining placentas of parents who delivered preterm, there was no clustering of microbial communities by obesity.[23] In contrast, a study in term parents found the placental microbiome of obese pre-pregnant parents to have less diversity, less microbial richness, and less abundance of taxa[24].

HMOs are considered a pre-biotic for infant gut bacteria, and thus altered composition could impact the gut microbiome. The milk microbiome and HMOs may be altered in the setting of obesity. In general, parents with elevated BMI have been found to have a less diverse milk bacterial community.[25] An elevated BMI has been associated with a higher relative abundance of *Staphylococcus*,[26–28] *Akkermansia*,[25,26] *Corneybacterium*,[28] *Granulicatella*,[28,29] and low abundance of *Lactobacillus*,[26,27] *Bacteroidetes*,[29] *Bifidobacterium*,[25,26] and *Streptococcus*[27] in the milk microbiome. However, there are some studies which have found no relationship between BMI and milk microbial composition.[30,31] One study found obese parents were more likely to be non-secretors (those who cannot produce fucosylated HMOs), and among non-secretors, there was increased sialyl-lacto-N-tetraose b (LSTb) and fucosyl-disialyl-lacto-N-hexaose (FDSLNH) in overweight parents compared to normal weight parents.[32] Lacto-N-pentose II/III, and lacto-N-fucopentose I were associated with parent pre-pregnancy BMI.[31] Fucosylated HMOs are an important energy source for infant gut *Bifidobacterium and* may impact the development of the infant gut.[33]

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Parental overweight/obesity status has also been associated with alterations to the infant gut microbiome. One study found meconium samples of infants born to parents who were obese pre-pregnancy, had less Firmicutes and increased Proteobacteria compared to infants born to parents with normal pre-pregnancy BMI.[14] When examining infants of overweight parents up to 12 months, diversity indices of the gut microbiome were increased in the overweight group compared to the normal weight group.[34] However, the evidence of changes in infant gut bacterial abundance are mixed. In a study examining infant gut bacterial abundance at the genus level, infants from overweight parents had greater abundance of *Salmonella, Serratia* and *Coprobacillus*; however, significance was only achieved with unadjusted p-values.[34] Another study found mode of delivery altered the findings. There were no associations of pre-pregnancy BMI and the infant gut microbiome in the Cesarean-delivered infants, but in the vaginal birth delivered group, there was increased *Bacteroides fragilis, Escherichia coli*, *Veillonella dispar*, *Staphylococcus,* and *Enterococcus*.[35] Infants of overweight parents had less butyric acid-producing bacteria compared to normal-weight parents.[34] These findings suggest infants of overweight/obese parents begin with pathogenic bacteria with less butyrate production. Butyrate has been considered the optimal energy source for colonocytes, and limited source of this could impact intestinal health.[36]

UNDERWEIGHT

The effects of parental underweight on the parental and infant microbiome are less well-defined than that of obesity and overweight. There is animal data to suggest that parental undernutrition alters the microbiome. A study of pregnant cattle demonstrated decreased placental microbial diversity in the setting of feed restriction in late gestation.[37] Furthermore, feed restriction of pregnant ewes results in an altered relative abundance of gut microbial communities.[38] Feed restriction of ewes additionally results in decreased colonic microbiome diversity and increased relative abundance of *Peptococcaceae* and decreased relative abundance of *Ruminococcus*.[39] *Peptococcacea* has been associated with higher high-density lipoprotein cholesterol,[40] whereas *Ruminoccocus* plays a key role in complex carboydrate degradation[41] and may be important to maintaining colonic health as decreased abundance of Ruminococcaceae has been implicated in inflammatory bowel diseases[42,43] and antibiotic associated diarrhea.[44]

Evidence from individuals with anorexia has demonstrated decreased alpha diversity[45] and lower amounts of total bacteria and obligate anaerobes in fecal samples when compared to well-nourished individuals.[38] Reduction of energy-balance and nutrient load in the diet results in an increase in Firmicutes and a decrease in Bacteroidetes; similar to obese adults.[46] This could be plausible as these bacteria facilitate energy usage, and in a starved state this is critical.

Additionally, evidence from young children with severe undernutrition demonstrates less mature gut microbiomes that may promote excessive weight loss.[47] Children with malnutrition have lower richness and increased abundance of Proteobacteria, including pathogenic *Klebsiella* and *Escherichia* and lower abundance of *Bacteroidetes* when compared to healthy children.[48] Reduced diversity and increased relative abundance of *Acidaminococcus* is also reported among children with undernutrition.[49] Taken together, it is plausible and likely that undernutrition in pregnancy results in distinct changes to the parental microbiome which will affect offspring, though additional studies focusing on the microbiome of undernourished individuals are needed.

GESTATIONAL WEIGHT GAIN

The role of gestational weight gain in pregnancy on the parental gut microbiome has been controversial. United States Preventative Task Force has differing recommendations of weight gain depending on pre-pregnancy BMI with less weight gain (11 to 20 lb) for obese pregnant parents and more weight gain (28 to 40 lb) for underweight pregnant parents.[50] The recommended amount of weight gain in pregnancy was associated with increased *Bifidobacterium* compared to those with excessive weight gain.[16] However, when controlling for pre-pregnancy BMI, there were no differences in diversity with excess gestational weight gain,[17,51]. There have been changes in bacterial abundance with excess gestational weight gain. Increased gestational weight gain was

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associated with decreased *Prevotella* and *Dialister* in the third trimester.[17] *Dialister* has been associated with insulin sensitivity.[16] For those with normal pre-pregnancy BMI, when they had excessive gestational weight gain, there was an increased in Firmicutes and Bacteroidetes phyla,[51] but in those who were obese prior to pregnancy who had excessive weight gain, there was an increase only in Bacteroidetes.[51] This could be due to preexistence of high quantities of Firmicutes in the context of obesity.

The milk and placental microbiome also appear to be altered by gestational weight gain in pregnancy. Multiple studies have found that with increased gestational weight gain there was an increase in milk microbiome alpha-diversity.[52,53] Independent of parental obesity, increased gestational weight gain has been associated with increased abundance of *Staphylococcus*,[25–27] decreased *Streptococcus*,[53] and decreased *Bifidobacterium*.[26,53] *Staphylococcus* has been associated with the pro-inflammatory state of obesity,[54] and may contribute to the infant's risk for future metabolic diseases. Additionally, the placental microbiome in parents who deliver preterm clusters by excess gestational weight gain.[23] For those with excessive gestational weight gain, there are decreased species richness, decreased Proteobacteria, increased Actinobacteria, increased Firmicutes, and increased Cyanobacteria.[23] These findings are thought-provoking, as excessive gestational weight gain and decreased placental species richness have both been associated with increased risk of preterm birth,[55] and thus provide a potential mechanism that could be amenable to intervention.

Increased gestational weight gain in pregnancy has been associated with changes in the offspring microbiome from the neonatal period to adulthood. Gestational weight gain has been associated with decreased *Akkermansia* abundance at 1 month[34], increased diversity at 6 months[34], increased enrichment of microbial glucose and glycogen degradation pathways, and increased microbial phenylalanine, cysteine/serine, folate, thiamin, biotin, and pyridoxine synthesis pathways at 8 months.[56] These studies suggest gestational weight gain impacts the infant's gut bacterial role in energy storage, which could have implications on their risk for obesity and metabolic syndrome. Another study examining women 19 to 44 years old whose parents demonstrated excess gestational weight gain, found those who exposed to excess gestational weight gain had increased visceral adiposity and increased fecal *Acidaminooccus*, a bacteria associated with adiposity, in adulthood.[57] These findings suggest that adiposity even in adulthood may have fetal and microbial origins.

BODY COMPOSITION

Given pre-pregnancy BMI is a flawed proxy for adiposity,[58] nutritional scientists are moving toward the use of body composition. Body composition examines fat-mass (brown, subcutaneous, visceral fat) versus fat-free mass (muscle, organs, or bone), or skeletal muscle mass. Body composition can be studied using various technologies that vary in cost and resolution. Increased white visceral adipose tissue in pregnancy releases pro-inflammatory cytokines and free fatty acids which can alter the epigenome of fetus' muscle, liver, and adipose tissue.[49] These adaptations can increase the child's risk of metabolic syndrome and nonalcoholic fatty liver disease. As mentioned previously, parents with increased BMI have also been found to have significant differences in the human milk oligosaccharide profile,[8,34] but no studies have directly examined the relationship between parental body composition and human milk oligosaccharide concentrations.

Given the release of free fatty acids and the changes in mother's own milk human milk oligosaccharide composition, there are likely alterations in the parental and infant microbiome. However, this has not been well-studied. In a study examining adults with type 2 diabetes, adults with a greater lean tissue index (lean tissue mass divided by height squared) had a higher ratio of Firmicutes to Bacteroidetes phylum.[59] In a rat model giving a high protein diet versus a fat and sucrose diet, a high protein diet was associated with decreased fat mass, increased alpha-diversity, increased abundance of *Lactobacillacea* and *Bifidobacterium* in the parent.[60] In the offspring of the high protein mice, they found decreased fat mass in both male and female mice, but sex-dependent differences in the microbiome.[60] Both male and female offspring had differences in beta-diversity, but in males there was also increased alpha-diversity, increased *Bifidobacterium*, increased

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Muribaculaceae, and decreased *Lachnospiraceae*.[60] In an observational study examining 140 pregnant parents, increased fat mass in pregnancy was positively associated with increased *Akkermansia*, *Blautia*, and *Bilophila*.[17] *Bilophila* is a bile-resistant bacillus that expands in the presence of dietary fats and has been associated with increased intestinal inflammation in a mouse model.[61] There have been no other studies to date examining parental body composition in pregnancy and the microbiome, and further research is needed to define the role of body composition on the microbiome and better understand the microbial mechanisms for parental and neonatal metabolic changes.

GESTATIONAL DIABETES

Gestational diabetes is a hyperglycemic state that occurs during pregnancy. Gestational diabetes is one of the most common complications in pregnancy that has been increasing in prevalence alongside both the obesity epidemic and increasing parental age at time of conception.[62] To meet the demands of the developing fetus, a state of transient hyperinsulinism is necessary to store energy; however, some parents are unable to compensate for hyperinsulinism and develop hyperglycemia due to pancreatic beta-cell dysfunction.[63] There is evidence that an altered microbiome with decreased short-chain fatty acid producing bacteria, decreased amino acid degrading bacteria, increased Firmicutes to Bacteroidetes ratio, and increased gram-negative bacteria lead to gut inflammation, gut permeability, increased dyslipidemia, and insulin resistance.[64] Additionally, a Western diet (low fruits and vegetables, high sodium and fat) was associated with increased risk of gestational diabetes where as a Mediterranean diet (higher bread, cereal, legume, fish, and olive oil diet) was associated with a decreased risk of gestational diabetes.[65]

The parental microbiome is altered in the setting of gestational diabetes. One study found that the vaginal, oral, and intestinal microbiomes were distinctly different from the non-diabetic microbiome on Bray-Curtis distance analyses comparing compositional similarity.[66] They found the oral cavity had more Proteobaceria and less Firmicutes in gestational diabetes, but no significant abundance differences in the intestinal or vaginal microbiome.[66] In contrast, one study examining 502 pregnant parents's vaginal microbiomes found gestational diabetes was associated with vaginal dysbiosis.[67] In regards to the gut microbiome, some experts argue that the origins of gestational diabetes are microbial.[59,61–63] When comparing the gut microbiota of pregnant parents in the first trimester, those who developed gestational diabetes had increased Ruminococcaceae family,[68] butyrate-producing bacteria *Faecalibacterium*[69] and *Eubacterium*.[69] In the third trimester of pregnancy, gestational diabetes is associated with increased gut *Bacteroides*,[70] *Streptococcus* and Enterobacteriaceae family.[69] All of these bacteria are associated with gut inflammation.[71] Further studies are necessary to determine whether altered gut bacteria in the third trimester predict gestational diabetes earlier than the glucose tolerance test in the second trimester.

Gestational diabetes also alters the microbial signatures of infants of affected parents. In one study, Chinese infants of parents with gestational diabetes and normal BMI were found to have decreased alpha diversity of meconium, as well as altered Firmicutes [72] and Proteobacteria at the phylum level[72]. Another study examining meconium from a similar population of parents with gestational diabetes found at the genus level altered *Prevotella*,[66] *Streptococcus*,[66] *Bacteroides*,[66] and *Lactobacillus* abundances.[66] *Lactobacilus* is important in amino acid synthesis *de novo.* [59] The decreased abundance of *Lactobacillus* may have implications for protein metabolism in the newborn.

It is difficult to disentangle the role of pre-pregnancy BMI and gestational diabetes on the microbiome as many studies are under-powered to adjust for BMI or do not include more nuanced assessments of body composition. However, one study comparing the parental gut microbiome in parents with gestational diabetes compared to those who were normo-glycemic controlled for pre-pregnancy BMI found decreased *Clostridium* and *Veillonella* after controlling for pre-pregnancy BMI.[73] Another study examining parental milk microbiome changes with gestational diabetes and pre-pregnancy BMI demonstrated microbial differences when adjusting for gestational diabetes.[28] Nonetheless, when examining gestational diabetes' role on the milk microbiome for those parents without obesity, they did not find significant differences.[28] Another study examining the infant meconium microbiome of infants born to parents with gestational diabetes and healthy parents found

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gestational diabetes state to be a driver of Bacteroidetes, Firmicutes, and Proteobacteria after adjusting for first trimester parental BMI.*[74]* Alterations in Bacteroides have been associated with type 2 diabtetes [63]. Furthermore, another study found neonates of parents with gestational diabetes had decreased *Lactobacillus*, *Flavonifractor*, *erysipelotrichaceae*, and *Gammoproteobacteria* after adjusting for pre-pregnancy BMI.[75] These findings provide evidence that gestational diabetes independently alters the microbiomes of parents and infants.

PARENTAL DIET AND THE MICROBIOME

There is evidence to suggest that parental diet may play a critical role in shaping the microbiome in pregnancy and the neonate independent of parental body habitus. Parental dietary intake during pregnancy is associated with parental gut, vaginal, and milk microbiome composition.[21,76,77] Subsequently, the neonatal microbiome is influenced by parental diet. Potential mechanisms of transfer to the infant include via vaginal delivery, the placenta, or the amniotic fluid. The effects of the parental diet on the infant stool microbiome persist after delivery for at least 6 weeks[78] and have been found to be greater among infants delivered vaginally than via Cesarean section.[76] However, when examining the drivers of the infant microbiome up until 6 months, chestfeeding status was the primary driver rather than parental dietary intake.[79]

Fat intake is associated with microbial shifts in pregnancy. Saturated fatty acid intake has been positively associated with gut microbial Simpson diversity index in obese/overweight participants.[17] In normal weight subjects, increased monounsaturated and polyunsaturated fatty acids are associated with *Ruminococcus* and *Paraprevotella* abundance.[17] *Ruminococcus* has been previously positively associated with polyunsaturated fatty acid supplementation and plant-based diets. [16,80] In a study examining mother-infant pairs in the Mediterranean, parental lipid intake has been associated with decreased Bacteroidetes and increased Firmicutes relative abundance prior to delivery, consistent with findings in obesity.[76] In regards to the milk microbiome, increased intake of saturated fatty acids and monounsaturated fatty acids were inversely related to the relative abundance of Corneybacterium in American parents.[29] However, the study was unable to look at the lipid profiles of the milk itself to see if this impacted the milk fat composition.[17] Fat intake has also been directly associated with changes in the infant microbiome.[78] A parental diet high in total lipids, saturated fatty acids, and mono-unsaturated fatty acids has been associated with enrichment of Firmicutes phylum and depletion of Proteobacteria phylum in infant meconium.[76] Infants of parents with high fat intake during pregnancy were found to have lower *Bacteroides* that persisted from birth to 6 weeks of age.[78] This effect was not modified by pre-pregnancy BMI and gestational diabetes, suggesting that parental diet may be a primary parental driver of the infant microbiome.

Fruit and vegetable consumption also influences the parental microbiome. Particularly, a Mediterranean diet of higher plant and limited animal protein appears to be influential. [81–83] Parents who consume vegetarian diets have lower relative abundances of *Collinsella*, *Holdemania*, and *Eubacterium* but increased abundance of *Roseburia*, and *Lachnospiraceae* compared to their omnivore counterparts in the gut microbiome during the second trimester of pregnancy.[81] *Lachnospiraceae* break down polysaccharides to short chain fatty acids and have been associated with people who practice a vegetarian diet.[72,84] A study examining adherence to a Mediterranean diet throughout pregnancy in Hawaiian parents found increased parental gut microbiome diversity and increased abundance of bacteria that produce short chain fatty acids.[82] A predominantly plant/fish protein diet also alters the milk microbiome. In a primate study providing a "Mediterranean diet" compared to a "Western diet" (high animal protein, high sodium, high sugar), they found the mammary tissues had 10-fold higher abundance of *Lactobacillus* with the Mediterranean diet.[83] A potential mechanism for these alterations in the milk microbiome may be through the entero-mammary pathway where gut bacteria are transmitted to the mammary gland by dendritic cells.

Parental fruit and vegetable intake has been frequently associated with infant gut microbial changes. When examining infant meconium, parental dietary fiber and vegetable protein intake is negatively associated with the relative abundance of *Coprococcus*, *Blautia*, *Roseburia*, *Ruminococcaceae*, and Lachnospiraceae families.[76] This suggests a more positive microbial profile as *Blautia* has

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been associated with increased visceral adiposity in adults.[85] In a study of 39 2-month-old infants in Taiwan, parents with high fruit and vegetable consumption had a higher abundance of Propionibacteriales, Propionibacteriacea, *Cutibacterium*, Tannerellaceae, *Parabacteroides*, and *Lactococcus*.[86] In contrast, infants of parents who ate less fruits and vegetables had higher abundance of *Prevotella*, *Isobaculum*, Clostridia, Clostridiales, Lachospiraceae, *Hungatella*, *Lachnoclostridium*, Ruminococcacaea, *flavonifractor*, *erysipelatoclostridium*, Acidaminococcaceae, *Phascolarctobacterium*, *Megamonas*, Betaproteobacteriales, Burkholderiacea, and Suterella.[86] *Cutibacterium* has been found to degrade hexoses to produce propionate.[75] Propoionate consumption has been shown to be associated with less antigen presentation on dendritic cells associated with allergic disease in mouse models.[87] Another study found similar results at 6 weeks of age but found the effect of fruit intake to be modified by mode of delivery[88]; infants born by Cesarean section whose parents had a high fruit intake had increased odds of high *Streptococcus* and *Clostridium*. As the infant ages, there are more environmental drivers to the gut microbiome, but parental dietary intake appears to continue to play a role. One study found at 6 months when controlling for type of milk (mother's own milk versus formula), solid food introduction, mode of delivery, age, parental education, and race/ethnicity, infants of parents who ate more fruits and vegetables had increased *Lactobacillus*.[79] *Lactobacillus* has been associated with cellular immunity in infants and has been utilized as a probiotic supplement in atopic diseases with some success.[89,90]

Additionally, fish and animal protein sources have been associated with changes in the parental and infant gut microbiome. Animal protein intake in pregnancy was positively associated with parental gut Shannon diversity index.[17] Low processed meat intake is positively associated with *Lactobacillus* abundance in the gut[79] and the vagina;[21] and total animal protein intake has been positively associated with *Collinsella* abundance.[17] In regards to the infant microbiome, one study found that higher parental animal protein intake is associated with higher abundance of *Veillonella*, *Escherichia/Shigella*, *Klebsiellla*, and *Clostridium* in infant meconium.[76] These bacteria have been associated with infant gut dysbiosis and inflammation in preterm infants.[91–94] Increased parental fish and seafood intake was positively associated with increased *Streptococcus* in six-week-old infants regardless of mode of delivery or parental BMI.[76]

Carbohydrates have also been associated with alterations in the parental and infant microbiome. Increased carbohydrates in pregnancy have been associated with increased Bacteroidetes in the parental gut microbiome prior to delivery.[76] Increased total carbohydrates and sugars in pregnancy was associated with improved vaginal health with a lower Nugent score.[21] In regard to the milk microbiome, increased total carbohydrates, disaccharides, and lactose were negatively associated with abundance of Firmicutes in lactating parents in the United States.[29] There is minimal information regarding parental diet and the milk microbiome, and further research is needed to characterize the microbial differences.

FUTURE DIRECTIONS – DIETARY AND PROBIOTIC INTERVENTIONS

Given the impacts of parental nutritional status and diet on the parental and infant gut microbiome, there have been efforts to improve parental and neonatal health outcomes through prebiotic, probiotic and dietary interventions. Unfortunately, these interventions have had varying levels of success.

Multiple randomized control trials utilizing probiotics have been conducted in overweight pregnant parents. There have been seven studies to date that have examined the use of probiotics in the prevention of gestational diabetes, and a recent meta-analysis conducted found that probiotics had no effect on the risk of gestational diabetes, cesarean section, gestational weight gain in pregnancy, or large for gestational age infants.[95] Another study providing fish oil and/or probiotic supplementation to overweight pregnant parents in early pregnancy to assess gestational weight gain and body composition found no significant differences in gestational weight gain or body composition[96], but supplementation did decrease *Ureaplasma* and *Prevotella*.[97] A recent meta-analysis found increased *Ureaplasma* abundance is associated with preterm rupture of membranes, preterm birth, chorioamionitis, and bronchopulmonary dysplasia, but evidence is low-quality.[98]

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Thus, probiotics decreasing the abundance of *Ureaplasma* may have benefits to pregnancy, but further large, randomized control trials are necessary.[99]To date, there is little evidence to support the use of probiotics in pregnancy.

Dietary and exercise interventions in pregnancy have been performed with variable success. In an animal model, methyl-donor nutrients (folate, vitamin B12, choline, methionine, betadine) provided in pregnancy and lactation to mice receiving a high fat diet lead to decreased cytokine expression, decreased colonic vitamin D receptor *(VDR*) signaling in pups.[100] VDR signaling impacts vitamin D metabolism.[81] The UPBEAT trial enrolled obese pregnant parents to participate in a low glycemic index diet plus physical activity and resulted in decreased skinfold thickness, gestational weight gain, improved metabolome in parents, and decreased infant subscapular skinfold thickness z-score at 6 months.[101] Another study examined if the use of a "HealthyMoms" smartphone app in pregnancy would improve gestational weight gain, glycemia, and insulin resistance,[102] and did not find any significant differences in clinical outcomes. However, the parents did have improved healthy eating scores post-partum.[82] Further research is needed to examine the microbial changes to the mother and neonate following nutritional interventions.

GAPS IN THE LITERATURE

Better understanding of how parental factors including BMI, body composition and diet affect the infant microbiome is needed. Longitudinal studies following from pre-pregnancy and early pregnancy through the period of exclusive chestfeeding would allow for thorough characterization of interactions between parental health, the parental microbiome, and infant health and microbiome. Multi-omic studies evaluating metabolomic and microbiome signatures would allow for a more thorough understanding of interactions between host metabolism, the microbiome and microbial metabolism. Another large gap in the literature is the effect of parental undernutrition including macronutrient and micronutrient deficiencies, low BMI, and inadequate gestational weight gain on both the parental and infant microbiome. Additionally, current methods for assessing parental diet are inadequate as 24 hour recalls, food frequency questionnaires, and other survey methods may not accurately capture dietary intake and quality. As the double burden of malnutrition continues to increase among pregnant people globally, a clear understanding of the effects of malnutrition on the microbiome and infant outcomes is necessary to identify novel targets for intervention to optimize outcomes and development among offspring.

CONCLUSIONS

Parental metabolic factors regarding adiposity, lean mass accretion, insulin resistance, gestational weight gain, and diet all have microbial implications to the mother, the milk produced, and the offspring. Dietary differences during pregnancy appear to be one of the largest drivers of the parental microbiome but are the most difficult to study reliably due to methodology utilized. There also is a paucity of literature on undernutrition's implications on the parental microbiome. Microbial shifts in the mother and their offspring can influence their risk for future metabolic diseases. Although interventions with probiotics in pregnancy have not been successful, dietary changes appear to have the most promise. Further research efforts should concentrate on multi-omic approaches and utilize dietary assessments throughout pregnancy and the lactation period, mother's own milk composition, and the parental and neonatal microbiome in vulnerable populations to provide precise nutritional and microbiome directed interventions.

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