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# Charting the Maternal and Infant Microbiome: What Is the Role of Diabetes and Obesity in Pregnancy?

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## Abstract

**Purpose of Review—**The purpose of this review is to summarize the evidence on whether diabetes, obesity, and related metabolic derangements during pregnancy are associated with the maternal and infant microbiomes, and to identify gaps in the literature and offer guidance on future research on this topic.

**Recent Findings—**We found circumstantial evidence from four observational studies that the maternal gut microbiome was associated with either pre-pregnancy body mass index, gestational weight gain, gestational diabetes, and/or related metabolic biomarkers in pregnancy; we did not identify any studies that examined whether the vaginal microbiome varied according to these metabolic parameters. Maternal diabetes (in one study) and pregnancy weight status (in three studies) were found to be associated with the infant offspring gut microbiome, although some associations only appeared in certain cohort strata. Patterns of association across both maternal and infant microbiome studies, however, lacked consistency, which may owe to biologic or technical differences, or to the lack of control for important confounders or effect modifiers (e.g., delivery mode in infant microbiome studies).

**Summary—**Metabolic diseases in pregnancy, such as diabetes and obesity, may be associated with the maternal and infant microbiomes, but there is a need for large prospective studies of mother-child dyads from diverse racial and ethnic backgrounds to determine the direction and potential causal nature of these associations. These studies should include serially collected biospecimens, standardized workflows that conserve microbial DNA and RNA, and rich data on clinical outcomes and environmental, lifestyle, and genetic risk factors for obesity and diabetes.

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**Keywords**

Gestational diabetes; Pregnancy; Diabetes; Obesity; Gestational weight gain; Microbiome

## Introduction

Diabetes, obesity, and related metabolic disorders characterized by chronic inflammation and impaired glycemic control are rapidly increasing among pregnant women in the USA and throughout the globe [1–6]. The rise in the prevalence rates of obesity and diabetes (henceforth also referred to as *diabesity*) in pregnancy is a serious public health concern as it not only elevates future cardiometabolic disease risk in mothers [6, 7], but also places offspring at higher risk of developing chronic diseases such as obesity [8–10], metabolic syndrome [11], non-alcoholic fatty liver disease [12], type 2 diabetes [13], and possibly even autism [14].

The mechanism by which maternal diabetes and obesity portends offspring risk for chronic diseases is not well understood. One emerging hypothesis is that the effects may be mediated through alterations in the maternal microbiome during pregnancy that is shared with the newborn either during gestation, delivery, or after. In this article, we will review the evidence that diabetes, obesity, and related metabolic derangements during pregnancy impact the maternal and infant microbiomes. Additionally, we will identify gaps in the literature and offer guidance on future research.

## Appreciating the Gut and Vaginal Microbiome

The tens of trillions of body-habitat-associated microbial communities (microbiota) and their genes (microbiome), collectively known as the human microbiome, are an essential part of human life. Among the human body habitats, the gut and vagina host two microbial ecosystems crucial to health.

The adult gut contains 500–1000 species of mostly anaerobic bacteria that belong to only a few phyla that increase in density and diversity from the proximal to distal gut [15, 16]. The gut microbiome is involved in extraction of energy and production of short chain fatty acids from otherwise indigestible carbohydrates [17, 18], biosynthesis of vitamins [19], release of gut hormones [20], maintenance of the gut barrier function [21], and training of the immune system [22] among other functions.

The vaginal microbiome is less diverse than the gut microbiome, as it is generally dominated by lactobacilli [23]. Through fermentation of glycogen, certain *Lactobacillus* species indigenous to the human vagina (i.e., *L. iners, L. crispatus, L. jensenii*, and *L. gasseri)* produce lactic acid to maintain a low vaginal pH and prevent pathogenic bacteria from invading the birth canal [23]. Lactobacilli also outcompete other bacterial species and produce a substance called bateriocin that can directly kill unwanted bacteria [23]. In addition to preventing bacterial invasion, there is evidence that the vaginal microbiome may play key roles in hormone secretion, timing parturition, and, importantly, microbial seeding of the newborn during natural birth [24].

## Pregnancy and the Microbiome

Pregnancy is marked by numerous metabolic changes that support maternal and fetal health and development. Koren and colleagues were the first to use high-throughput sequencing to examine whether the gut microbiome changes during pregnancy [25]. They followed 91 healthy Finnish women through pregnancy and found that compared to first trimester stools, third trimester stools differed phylogenetically and had lower microbial species richness [i.e., number of operational taxonomic units (OTUs)]. In particular, third trimester stool had a greater relative abundance of proinflammatory *Proteobacteria* and *Actinobacteria* [25]. The investigators then inoculated (perorally) gnotobiotic mice with gut microbiota from stool collected in the third vs. first trimester. Compared to mice that received the first trimester microbiota, those receiving the third trimester microbiota gained more weight, became more insulin resistant, and had higher levels of inflammatory markers [25], suggesting that the gut microbiota may be responsible for pregnancy-related metabolic changes. In contrast, DiGulio et al. collected rectal swabs weekly from the end of the first trimester to delivery in 40 US women (11 of which delivered preterm) and observed only marginal decreases in alpha diversity (Shannon diversity index) over the gestational period ($p = 0.05$), and no changes to microbial beta diversity nor shifts in relative abundance of taxa [26]. Likewise, a study of 56 Tanzanian women (26 of whom received probiotic supplementation), in which monthly stools were collected from 12 to 24 weeks of pregnancy to parturition, found no significant changes in the gut microbiota beta diversity over the course of pregnancy; in this study, changes in alpha diversity or relative abundance of specific taxa with pregnancy were not reported [27]. The divergent study results may be attributable to differences in the following: (*i*) the frequency and method of sampling, (*ii*) the hypervariable regions amplified (*V1/V2* vs. *V3–V5* vs. *V4* in the three studies, respectively), or (*iii*) the size and composition of the cohorts studied.

It is well known that the vaginal microbiome during pregnancy is distinct from the non-pregnant vaginal microbiome [28], and it has also been purported to change through the course of gestation. Of the four major longitudinal studies on the vaginal microbiome in pregnancy [26, 28–30], two [28, 30] found decreases in species diversity and richness, with concomitant increases in *Lactobacillus* over gestational time. Three of the four also reported movement between distinct vaginal community state types [26, 28, 30]. While there has been speculation that the vaginal microbiome is colonized by the maternal gut microbiome (via the rectum) toward the end of pregnancy [31], there is limited empirical support for this hypothesis [27].

Given the lack of consensus about programmatic changes to the maternal microbiome during gestation, there is need for large prospective studies that use consistent sample collection, storage, and processing. These studies should be conducted in diverse populations, as race and ethnicity have been shown to modify microbiome composition [32], and they should consider going beyond amplicon sequencing, e.g., shotgun metagenomic sequencing, to improve resolution and examine functionality of distinct microbial ensembles. Such studies will help to not only illuminate whether there are natural pregnancy-related changes to the gut and vaginal microbial communities and their functions but also whether these changes are adaptive for the health of mothers and their offspring.

## Diabetes and Obesity in Pregnancy and the Maternal Microbiome

Although no investigations have directly examined the association of gestational diabetes with the maternal body-habitat-associated microbiota *during* pregnancy, data exist on the associations of gestational diabetes with the postpartum maternal gut microbiota [33••]; weight status and metabolic hormones with the maternal gut microbiota during pregnancy [33••, 34••]; and pre-pregnancy body mass index (BMI) and gestational weight gain with the maternal gut microbiota during pregnancy [35, 36] (Table 1).

Fugmann and colleagues aimed to examine whether gestational diabetes was associated with postpartum gut microbiota composition and structure by collecting stools 3 to 16 months post-delivery from 42 women who in their most recent pregnancy had gestational diabetes and 35 women who had experienced normoglycemic pregnancies (controls) [33••]. The investigators found no differences in alpha diversity (species richness) or beta diversity (phylogenetic distance), but they did note lower relative abundance of *Firmicutes* in the stool of women with previous gestational diabetes. Lower relative abundances of several OTUs in the phylum *Firmicutes* have also been observed in patients with type 2 diabetes [37–39]; however, metabolic functionality of genera and species within the *Firmicutes* phylum vary markedly, making direct comparisons difficult.

Maternal weight and metabolic-hormone status have further been examined in relation to the gut microbiome of women during pregnancy. Gomez-Arango et al. collected blood and stool samples from 29 overweight and 41 obese pregnant women at 16 weeks of gestation [34••]. While not statistically significant, the gut microbiome of obese (vs. overweight) women tended to have lower microbial richness (number of unique OTUs) and evenness (relative prevalence of the various OTUs)—two measures of alpha diversity. There was no difference in beta diversity, or phylogenetic distance, between the stool microbiota of overweight vs. obese women. However, comparing microbial composition at the phylum level, obese (vs. overweight) women had significantly higher relative abundances of *Firmicutes* and *Actinobacteria*, and lower of *Tenericutes*. At the family level, maternal obesity vs. overweight was associated with greater abundances of *Lachnospiraceae* and *Rikenellaceae.* The authors also reported many significant univariate associations of metabolic biomarkers with taxonomic relative abundances, e.g., C-peptide and fasting insulin with *Coriobacteriaceae* (family) and *Collinsella* (genus) (see Table 1); however, it is not clear if these associations were independent of adiposity as they were not adjusted for maternal BMI. Although the results indicate associations of maternal weight status and metabolic markers in pregnancy with gut microbiome composition, the cross-sectional nature of this study leaves the direction of these associations uncertain.

Two studies have examined the impact of pre-pregnancy body weight and weight gain during pregnancy on the maternal gut microbiome [35, 36]. A prospective Finnish study ($n$ =54), analysis using FISH-FCM and qPCR, reported higher pre-pregnancy BMI was associated with higher concentrations of the genera *Bacteroides, Clostridium*, and *Staphylococcus*. Excess gestational weight gain was positively associated with *Bacteroides* and negatively with *Bifidobacterium* in the third trimester [35]. A cross-sectional study from Spain ($n$ = 50) that analyzed stool using qPCR at 24 weeks found reduced concentrations of

*Bacteroides* and *Bifidobacterium*, and increases in *Staphylococcus*, *Enterobacteriaceae*, and *Escherichia coli* in 16 women who were overweight before pregnancy compared to 34 women who were normal weight [36]. The authors further showed that excessive weight gain was positively associated with *E. coli, Clostridium leptum, Staphylococcus*, and *Enterobacteriaceae* and inversely associated with *Bifidobacterium* and *Akkermansia muciniphila*, two phylotypes that experimental murine models have implicated in etiology of obesity [40, 41] and diabetes [42•, 43]. The Finnish and Spanish studies on body weight in pregnancy did not use high-throughput sequencing techniques and thus were unable to examine associations with more contemporary measures of alpha and beta diversity (Table 1).

## Maternal Microbiome and Metabolic Disorders in Pregnancy: Potential Mechanisms

The associations of metabolic derangements and altered pregnancy-associated gut microbiome composition may be bidirectional and governed by several mechanisms. Short chain fatty acids, for example, are produced by microbial fermentation of undigested dietary polysaccharides in the colon [17, 18], and the quantity and type of fatty acids produced may be partially determined by the composition of the gut microbiome [44•]. In addition to providing energy, short chain fatty acids may stimulate intestinal L cells to secrete glucagonlike peptide 1 [20]. This in turn increases glucose-stimulated insulin secretion [45] and may decrease systemic inflammation [46, 47].

The gut microbiota composition may additionally regulate intercellular tight junctions in the intestinal mucosa [21], thereby inhibiting the translocation of inflammatory endotoxins like lipopolysaccharides from the lumen into circulation [48] (Fig. 1). Compared to non-pregnant women, pregnant women have been shown to have greater intestinal permeability, as measured by lactulose/mannitol tests [49]. This may be further modified by metabolic status in pregnancy. Zonulin is a novel protein that influences intestinal barrier function by maintaining the tight barrier junctions between goblet cells [50]. Circulating zonulin and lipopolysaccharides have been positively associated with obesity, measures of inflammation, and deranged glycemia [51–53]. Among overweight pregnant women, those with greater serum zonulin have been shown to have lower microbial species richness, higher abundance of *Bacteroides* and *Blautia*, and lower abundance of *Faecalibacterium prausnitzii*, a short chain fatty acid producer [54].

Thus, an imbalanced gut microbiota associated with metabolic disorders in pregnancy may induce inflammation and glycemic derangements via altered short chain fatty acid production, regulation of gut hormone secretion, and/or intestinal barrier function (Fig. 1). Interestingly, the widely used diabetes drug metformin may help mitigate gut dysbiosis associated with obesity and diabetes. In human [55, 56] and mice [42•, 57–59] studies, metformin has been positively associated with the relative abundance of *A. muciniphila* and short chain fatty acid-producing bacteria in the gut microbiota. Based on this small but growing literature base, it is tempting to postulate that metformin—a drug with no known

risks for the mother or fetus—could not only help ameliorate diabetes-associated dysbiosis in pregnancy, but also reduce transmission of diabesogenic bacteria to the newborn.

## Seeding and Development of the Infant Gut Microbiome: Mom Matters

The mother is the source of the newborn's first microbiota, either through low biomass exposure before birth, the rich inoculum of vaginal and gut microbiota during labor, or the skin microbiota during C-section delivery [60]. As such, it is conceivable that a maternal microbiome associated with diabetes or obesity during pregnancy could potentially modify the mother-to-newborn transfer of microbiota.

Maternal diabetes or obesity could influence the maternal-offspring exchange of bacteria via the maternal-fetal interface. However, it is unclear whether such an exchange occurs. For example, in a recent study, Lauder and colleagues found no difference between the bacterial DNA from placental samples and negative controls, indicating that the "placental microbiome" identified in previous studies [61–64] may have been due to contamination [65•]. Thus, while we are aware of studies showing associations of gestational diabetes [66] and maternal pregnancy weight gain [62] with bacterial DNA in the placenta, we have focused our review on less bias-prone studies that have examined the impact of metabolic disorders in pregnancy on mother-to-newborn transmission of the rich microbial ecosystem at birth.

After seeding at birth, an infant's gut microbiome typically undergoes three distinct phases of development [67]. During the first month, it is dominated by facultative aerobic *Enterobacteriaceae*, after which strict anaerobes, namely *Bifidobacterium, Bacteroides*, and *Clostridium*, begin colonizing the gut. Between months 6 and 24, a diverse mixture of *Clostridiales* takes over the gut microbiota due to the increased intake of solid foods. This shows a general progression from an aerobic to an anaerobic-dominated microbiome. At around 2 years of age, the infant gut stabilizes, approaching a typical adult microbiota profile.

The maturation of the newborn gut microbiota is greatly influenced by mode of delivery. Bokulich and colleagues recently conducted an eloquent study that showed that immediately after birth, cesarean-delivered newborns have a gut microbiome with greater phylogenetic diversity, richness, and evenness compared to vaginally delivered newborns. However, this trend reversed from 8 months to 2 years, with cesarean-delivered infants having lower diversity and richness [67]. Moreover, in this study, microbiota maturation stagnated in cesarean-delivered infants between 6 and 24 months of age compared to vaginally born infants [67]. Bokulich et al., along with other studies on this topic [68, 69], further observed that colonization by *Bacteroidetes* was reduced in C-section babies for at least the first year of life. Irrespective of delivery mode, breastfeeding also impacts the infant gut microbiome [68], but it is increasingly clear that delivery mode is the predominant driver of the first colonizers of the infant gut.

## Maternal Diabetes and Obesity and the Infant Microbiome

Several studies have reported on the association of maternal metabolic disorders in pregnancy with measures of the infant gut microbiome composition and structure (see Table 2).

Collado and colleagues enrolled 42 Finnish pregnant women (16 overweight) and collected stool samples from their infants at 1 and 6 months after birth for analysis using FISH-FCM and qPCR [70]. Compared to normal weight mothers, overweight mothers had children with higher stool concentrations of *C. leptum, Clostridium histolyticum*, and *Staphylococcus* as well as a lower concentration of *Bifidobacterium* and *Clostridium perfringens* at 6 months of age and lower *Bacteroides* at 1 month. Excessive pregnancy weight gain was associated with a lower stool concentration of *Bacteroides* and a higher concentration of *C. histolyticum* at 1 month and lower concentrations of *Bifidobacterium* and higher concentrations of *Staphylococcus aureus* and *C. histolyticum* at 6 months of age. Infants of overweight mothers and mothers with excessive weight gain during gestation were also more likely to have *C. difficile* in their stool at 6 months. Measures of infant stool alpha diversity and beta diversity were not examined.

In a 2014 study using 454 pyrosequencing, Galley et al. examined associations of maternal pre-pregnancy BMI and pregnancy weight gain with the gut microbiota composition and structure of 77 infants (26 from obese mothers), aged 18 to 27 months at the time of stool collection [71••]. The authors stratified participants by socioeconomic status (SES) and found that in the high SES group ($n = 47$), infant stool microbiota clustered (beta diversity) significantly with pre-pregnancy BMI status, and infants born to obese ($n = 14$) vs. non obese mothers ($n = 33$) had greater alpha diversity, as indicated by the Shannon index and measures of microbial richness and evenness. Among the high SES group, children born to obese mothers (vs. non obese mothers) had stool with higher relative abundances of the genera *Parabacteroides*, *Oscillibacter*, and an unclassified genus of the order *Bacteroidales*, and lower relative abundances of the genera *Blautia* and *Eubacterium.* However, the authors did not stratify analyses by delivery mode, nor did they report if C-section rates differed by SES status; delivery mode could be an important effect modifier of the maternal BMI-infant microbiota association.

The impact of maternal diabetes in pregnancy on the microbiome of meconium, i.e., the first newborn stool after birth, has also been explored [72]. In a study of 23 neonates (5 from mothers with adult diabetes diagnosed before pregnancy, 5 from mothers with gestational diabetes, 4 from mothers with subclinical diabetes, and 9 from mothers with no diabetes), the meconium of newborns born to mothers with pre-gestational and gestational diabetes clustered (beta diversity) significantly differently from the meconium of newborns born to mothers without diabetes. The meconium of neonates born to mothers with diabetes diagnosed before pregnancy showed higher alpha diversity than the meconium from neonates born to non-diabetic mothers or to mothers who had gestational diabetes. Furthermore, the diabetes group had meconium with significantly higher abundance of *Bacteroidetes* (phylum), *Lachnospiraceae* (family), and *Parabacteriodes* (genus), and lower *Proteobacteria* (phylum). The authors also reported no differences in newborn meconium

microbiota composition or structure by delivery mode, which raises the possibility that maternal diabetes may alter the fetal acquisition of microbiota from amniotic fluid before birth. However, this hypothesis will need to be substantiated by future studies.

In a more recent study, Mueller et al. aimed to examine whether the association between maternal body weight status and infant microbiome was birth-mode specific by enrolling 74 neonates, 18 born vaginally (5 to overweight or obese mothers), and 56 by elective C-section (26 to overweight or obese mothers) [73••]. In the vaginal, but not C-section, delivered group there were statistically significant differences in beta diversity by maternal pre-pregnancy BMI. Among the vaginally delivered neonates, those born to overweight or obese mothers had stool enriched in *Bacteroides* (genus) and depleted in other taxa including *Enterococcus.* The stool of C-section neonates had minor differences in the *Bacteroides* genus, with higher levels in the normal weight group, although overall the relative abundance of *Bacteroides* in C-section born neonates was markedly lower than in vaginal born neonates. Furthermore, there were predicted metagenomic functional differences associated with pre-pregnancy BMI in the vaginally delivered neonates, but none in the C-section-delivered neonates, suggesting the impact of maternal body weight transpires through the vaginal/rectal inoculum at birth.

## Conclusions and Future Research Directions

Thus far, findings on how metabolic pathologies during gestation relate to the structure and composition of the human pregnancy-associated microbiome derive mostly from cross-sectional studies that are unable to discern temporal sequence, and associations with the infant microbiome come from small cohort studies with a single collection of stool, precluding analysis of the dynamic microbiome changes that occur during infancy. As yet, we are not aware of any observational studies that have examined the association of gestational diabetes with the maternal gut microbiota *during* pregnancy, or with the infant microbiome beyond the first stool (meconium). There is also a dearth of studies on the effect of obesity, excess gestational weight gain, or diabetes on the vaginal microbiome in pregnancy, which is a critical area for future research because of its role in seeding the infant offspring microbiome at birth.

Considering the growing prevalence of diabetes in pregnancy and the potential importance of the maternal microbiome for maternal and infant health, there is need for large longitudinal cohort studies of racially and ethnically diverse mother-child dyads with serial collection of maternal samples from early pregnancy through parturition, along with infant bio-specimens and clinical outcomes. Standardized workflows are needed to ensure comparability and more meaningful interpretations of information within and across studies. Ideally, workflows will include procedures to stabilize microbial DNA and RNA, which will facilitate higher resolution and specificity of microbial identification through whole genome sequencing and better understanding of microbial community functions—the metabolic activities and the end products resulting from these activities. These studies should also focus on rigorous collection of socio-demographic, lifestyle (e.g., diet, physical activity, sleep), and other environmental and genetic factors related to obesity and diabetes, as each

may have its own effect on the maternal microbiome and the microbiome of the offspring [74, 75].

It has been suggested that pregnancy-related changes to the maternal gut and vaginal microbiome are evolutionarily adaptive to promote the nutrition and development of the mother and fetus during pregnancy, and the child after birth [25]. Yet only with large prospective studies of pregnant women and their infants will we be able to test this hypothesis, to rigorously examine how the pregnancy-associated microbiome and the mother-to-newborn vertical transfer of microbiota are modified by metabolic disorders in gestation, and to assess whether these microbial alterations have consequences for the health of the mother and her offspring and if they can ultimately be targeted for interventions that improve public health.

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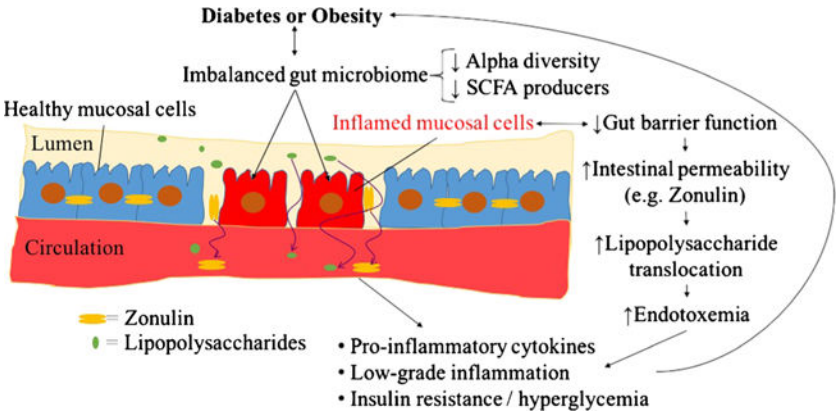


**Fig. 1.**

Simplified mechanism by which a diabetes- or obesity-associated gut microbiome may alter inflammation and glucose metabolism

**Table 1**

Human studies on the associations of obesity, diabetes, and related metabolic factors in pregnancy on the maternal gut microbiome

| Study | Exposure/outcome | Study population | Methods | Diversity measures | Microbial taxonomy measures | Limitations |
|---|---|---|---|---|---|---|
| Fugmann et al., 2015, Germany [33••] | Exposure: GDM in most recent pregnancy. Outcome: maternal gut microbiome at 3–16 months postpartum. | Inclusion: German women with GDM history ($n$ = 42) and without GDM history ($n$ = 35). Exclusion: pregnant women with substance abuse or chronic diseases requiring medication. | Design: cross-sectional. Sample: single stool. Analysis: *V4* sequenced via Illumina. Covariates: time since delivery adjusted for in beta diversity analysis and microbiome composition analysis. | Alpha diversity: no differences (Shannon, $p$ = 0.24; Chao 1, $p$ = 0.53; Simpson, $p$ = 0.11). Beta diversity: no differences (pMANOVA, $P$ = 0.1). | GDM (vs. no GDM) history: associated with a lower relative abundance of the phylum *Firmticutes*; no other associations remained statistically significant after adjustment for multiple comparisons. | Small sample size; single stool; collection 3–16 months after pregnancy. |
| Gomez-Arango et al., 2016, Australia [34••] | Exposures: maternal pre-pregnancy BMI and metabolic hormones. “Outcome” needs to be on its own line as it is in the other studies outcome: maternal gut microbiome at 16 weeks gestation. | Inclusion: obese ($n$ = 41) and overweight ($n$ = 29) pregnant Australian women. Exclusion: pregnant women with pre-existing diabetes or impaired glucose tolerance. | Design: cross-sectional study. Sample: single stool. Analysis: *V8* sequenced via Illumina. Covariates: no covariate control. Maternal age and gestational age were not associated with microbiome. | Alpha diversity: no differences (Shannon, $p$ = 0.098; Chao l, $p$ = 0.64). Beta diversity: no differences (pMANOVA, $p$ = 0.60). Diversity metrics were not assessed for metabolic hormones. | Positive (+) and negative (−) taxa correlations for: BMI: (+) *Fimticutes* (phylum); (+) *Actinobacteria* (phylum); (−) *Tenericutes* (phylum); (+) *Lachnospiraceae* (family); (+) *Rikenellaceae* (family). Insulin, HOMA-IR: (+) *Actinobacteria* (phylum); (−) *Tenericutes* (phylum); (+) *Coriobacteriaceae* (family); (+) *Collinsella* (genus). C-peptide: (+) *Fimticutes* (phylum); (+) *Collinsella* (genus); (+) *Coriobacteriaceae* (family); (+) *Rummococcaceae* (family). Leptin: (+) *Lachnospiraceae* (family); (+) *Rummococcaceae* (family). | Small sample size; single stool; no normal weight women; no adjustment for correlated metabolic factors. |
| Collado et al., 2008, Finland [35] | Exposures: pre-pregnancy BMI and weight gain in pregnancy. Outcome: maternal gut microbiome at 1st and 3rd trimester. | Inclusion: overweight ($n$ = 18) and normal weight ($n$ = 36) pregnant Finnish women. Exclusion: pregnant women with chronic or metabolic disease. | Design: prospective cohort study. Sample: stool at 1 st and 3rd trimesters. Analysis: FISH-FCM and qPCR. Covariates: no covariate control. | No diversity analyses were conducted. | Higher pre-pregnancy BMI associated with higher counts of *Bacteroides* (genera), *Clostridium* (genus), and *Staphylococcus aureus.* Excess gestational weight gain associated with higher counts of *Bacteroides* (genus) and lower *Bifidobacterium* (genus) in the 3rd trimester of pregnancy. | Small sample size; only two stool samples; FISH or qPCR did not allow for diversity analyses. |
| Santacruz et al., 2010, Spain [36] | Exposure: pre-pregnancy BMI and gestational weight gain. “Outcome” needs to be on its own line as it is in the other studies outcome: maternal gut microbiome at 24 weeks gestation. | Inclusion: overweight ($n$ =16) and normal weight ($n$ = 34) pregnant Spanish women. Exclusion: no exclusion criteria reported. | Design: cross-sectional study. Sample: single stool. Analysis: qPCR. Co variates: no covariate control. | No diversity analyses were conducted. | Higher pre-pregnancy BMI associated with lower ratio of *Bifidobacterium* (genus) to *Clostridium coccoides* and *Clostridium leptum* as well as lower counts of *Bacteroides* (genus) and *Bifidobacterium* (genus) and increases in *Staphylococcus aureus, Enterobacteriaceae* (family), and *Escheichia coli.* Excess gestational weight gain positively associated with *E. coli*, *C. leptum*, *Staphylococcus aureus*, and *Enterobacteriaceae* (family), but inversely associated with *Akkermansia muciniphihi*, *Bifidobacterium* (genus), and *Bacteroides* (genus). | Small sample size; single stool; qPCR did not allow for microbiota structure or diversity analyses. |

**Table 2**

Human studies on the associations of obesity, diabetes, and related metabolic factors in pregnancy on the infant gut microbiome

| Study | Exposure/outcome | Study population | Methods | Diversity measures | Microbial taxonomy measures | Limitations |
|---|---|---|---|---|---|---|
| Collado et al., 2010, Finland [70] | Exposure: pre-pregnancy BMI and weight gain in pregnancy. Outcome: infant gut microbiome at 1 and 6 months. | Inclusion: overweight ($n$ = 16) and normal weight ($n$ = 26) Finnish mothers and their infants. Exclusion: mothers with other chronic metabolic diseases (such as diabetes). | Design: prospective cohort study. Sample: infant stool at 1 and 6 months. Analysis: FISH-FCM and qPCR. Covariates: no covariate control. | No diversity analyses were conducted. | Overweight (vs. normal weight) pre-pregnancy BMI associated with: [FISH-FCM results] lower *Bacteroides-Prevotella* (genus) at 1 month, higher *C. histolyticum* at 6 months; [qPCR results] higher *C. leptum* and *S. aureus* and *lower Bifidobacterium* (genus) and *C. perfringens* at 6 months. Excess (vs. normal) gestational weight gain associated with [FISH-FCM results] lower *Bacteroides-Prevotella* at 1 month and higher *C. histolyticum* at 1 and 6 months; [qPCR results] lower *Bifidobacterium* (genus) and higher *S. aureus* at 6 months. | Small sample size; FISH or qPCR did not allow for microbiota structure or diversity analyses; lack of control or stratification by delivery mode. |
| Galley et al., 2014, USA [71••] | Exposure: pre-pregnancy BMI and weight gain in pregnancy. Outcome: infant gut microbiome at 18–27 months of age. | Inclusion: obese ($n$ = 26) and normal weight ($n$ = 51) US mothers and their infants. Exclusion: infants with major health conditions or toilet trained children. | Design: prospective cohort study. "Sample" Sample: single stool at 18–27 months. Analysis: bacterial tag-encoded FLX amplicon pyrosequencing. Covariates: stratified by SES (income and education). No associations of delivery mode, breastfeeding duration, or antibiotic exposure with microbiome community structure. | Alpha diversity: in the high SES strata, obesity was associated with higher alpha diversity (Shannon, $p$ = 0.026; Chao 1, $p$ = 0.043; unique OTUs, $p$ = 0.029). Beta diversity: in the high SES strata, obesity was associated with beta diversity (pMANOVA, $p$ = 0.044); clustering by PCoA. | In the high SES strata, higher pre-pregnancy BMI associated with higher relative abundances of *Parabacteroides* spp., *Oscillibacter* spp., and an unclassified genus of the order *Bacteroidales* and lower relative abundances of *Blautia* spp., and *Eubacterium* spp.; no associations were observed in the low SES strata. | Small sample size; lack of control or stratification by delivery mode; stool samples collected by mothers. |
| Hu et al., 2013, USA [72] | Exposure: diabetes in pregnancy. Outcome: meconium microbiome. | Inclusion: US mothers with pre-existing diabetes ($n$ = 5), GDM ($n$ = 5), and no diabetes ($n$ =13) and their infants. Exclusion: antibiotic use during pregnancy (unless for C-section) or clinical risks (such as HIV). | Design: prospective cohort study. Sample: meconium. Analysis: *V3-V4* region sequenced via 454 pyrosequencing. Covariates: no differences in microbiome composition by mode of delivery, maternal BMI in 1st trimester, gestational age, time of sample collection or baby's sex. | Alpha diversity: pre-existing diabetes group had higher alpha diversity than GDM or no diabetes (Shannon diversity index, $p$= le-3 and 0.08). Beta diversity: beta diversity for diabetes and GDM groups no different from the no diabetes group ($p$ = 0.006; $p$ = 0.0017). | Adult diabetes or GDM (vs. no diabetes) associated with lower relative abundance of *Proteobacteria* (phyla) and higher relative abundances of *Bacteroidetes* (phyla), *Lachnospiraceae* (family) and *Parabacteriodes* (genus). No significance was achieved when $p$ values were adjusted for multiple hypothesis testing. | Small sample size; single sample of meconium; no negative controls. |
| Mueller et al., 2016, Brazil [73••] | Exposure: pre-pregnancy BMI. Outcome: infant microbiome in first stool after meconium. | Inclusion: overweight ($n$ = 31; 5 vaginal delivery) and normal weight ($n$ = 43; 13 vaginal delivery) Brazilian mothers and their infants. | Design: prospective cohort study. Sample: first stool after meconium. | Alpha diversity: no differences. Beta diversity: in the vaginally delivered strata, pre-pregnancy BMI | In the vaginally delivered strata, higher pre-pregnancy BMI associated with higher relative abundance of genus *Bacteroides*, but lower of family *Xanthomonadaceae*, and genera *Pseudomonas, Acinetobacter, Hydrogenophilus*, | Small sample size; single sample of infant microbiome. |

| Study | Exposure/outcome | Study population | Methods | Diversity measures | Microbial taxonomy measures | Limitations |
|---|---|---|---|---|---|---|
| | | Exclusion: HIV, chronic metabolic diseases (such as diabetes), smoking, restrictive diets, or antibiotic use in the 3rd trimester. | Analysis: *V4* sequenced by Illumina. Covariates: stratified by delivery mode. No association of prenatal antibiotic use or breastfeeding with the infant microbiome. | associated with beta diversity (weighted UniFrac distance pMANOVA, $p < 0.001$) and clustering occurred (unweighted UniFrac PCoA). | and *Enterococcus*. In the C-section strata, minor differences in the *Bacteroides* genus with higher relative abundance in normal weight mothers, although the relative abundance of *Bacteroides* was low in both groups. | |

*GDM* gestational diabetes mellitus, *BMI* body mass index, *HOMA-IR* homeostasis model assessment of insulin resistance, *pMANOVA* permutational multivariate analysis of variance, *FISH-FCM* fluorescence in situ hybridization combined with flow cytometry, *qPCR* quantitative polymerase chain reaction, *PCoA* principle coordinate analysis