Gestational microbiome: metabolic perturbations and developmental programming

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Abstract

A growing body of research suggests that alterations to the human microbiome are associated with disease states, including obesity and diabetes. During pregnancy, these disease states are associated with maternal microbial dysbiosis. This review discusses the current literature regarding the typical maternal and offspring microbiome as well as alterations to the microbiome in the context of obesity, type 2 diabetes mellitus, and gestational diabetes mellitus. Furthermore, this review outlines the proposed mechanisms linking associations between the maternal microbiome in the aforementioned disease states and offspring microbiome. Additionally, this review highlights associations between alterations in offspring microbiome and postnatal health outcomes.

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Introduction

Since its completion in 2012, the Human Microbiome Project (HMP) has provided reference data on extensively characterized microbial communities in the human body, collectively known as the human microbiome. These microbial communities reside in various parts of the body, including the oral cavity, gut, skin, vagina, and more recently, possibly the placenta (Human Microbiome Project 2012, Aagaard et al. 2014, Kim 2015). Current research suggests that there is a ‘normal’ human microbiome and that alterations to the ‘normal’ microbiome, known as dysbiosis, are associated with chronic illness (Kim 2015). Alterations in the human gut microbiome have been implicated in the pathogenesis of a wide range of diseases, including obesity (Gomez-Arango et al. 2016) and type 2 diabetes mellitus (T2DM) (Barlow et al. 2015), which are the focus of this review.

The global prevalence of obesity and diabetes, including gestational diabetes mellitus (GDM), is defined as abnormal glucose tolerance with onset or first recognition during pregnancy (Sacks et al. 2012, Dabelea et al. 2014, Damm et al. 2016), is increasing. In 2016, the World Health Organization’s global health statistics reported approximately 50% of females globally are either overweight or obese, thereby increasing their risk of developing GDM (Chu et al. 2007, Zheng et al. 2017). Furthermore, GDM affects up to 5–20% of pregnancies (Ferrara 2007, Lavery et al. 2017) and is estimated to affect one in seven births internationally (International Diabetes Federation 2015). The global rise in the prevalence of obesity and GDM is a serious public health concern as it not only increases the risk of perinatal morbidity and mortality but also may contribute to long-term health complications in mothers and their offspring (Hillier et al. 2007, Zhu & Zhang 2016, Zheng et al. 2017). For instance, women with GDM have an increased risk for cesarean section (C-section) delivery, preeclampsia, and postpartum development of T2DM (Capula et al. 2014). Furthermore, the offspring of women with GDM are at an increased risk of complications such as congenital defects, fetal macrosomia, preterm birth, and metabolic dysfunction (Hillier et al. 2007, Catalano et al. 2012). Later in life, these offspring are more likely to develop obesity, insulin resistance, early onset T2DM (Kubo et al. 2014), metabolic syndrome (Boney et al. 2005), atopic dermatitis, and allergen sensitivity (Kumar et al. 2009). Although a link between maternal GDM and adverse maternal–fetal outcomes has been established in the literature, the mechanisms underlying such maternal metabolic derangement and fetal programming are not yet fully understood. More recently, a greater focus has been placed on understanding how alterations to the maternal microbiome may impact fetal programming and ultimately infant microbiome and health outcomes. In particular, maternal obesity and GDM have been linked to the altered maternal microbiome, which has been implicated in adverse maternal–fetal outcomes, such as preterm birth (Ardissone et al. 2014, Bassols et al. 2016, Gomez-Arango et al. 2016, Pelzer et al. 2017).
Furthermore, changes in the bacterial composition of the maternal microbiome have been shown to impact the bacterial composition of offspring meconium, which is used as a representative for the neonatal microbiome (Jimenez et al. 2008). While recent research suggests links between maternal metabolic derangement, alterations in maternal–fetal microbiome, and offspring health outcomes via fetal programming, this field of research warrants further attention.

This review will discuss the current literature on maternal–fetal microbiomes, focusing on the associations between maternal microbiome and health and how these influence the microbiome and health of the offspring. This review will also highlight the relationship between maternal microbiome and health in the context of maternal metabolic perturbations, such as obesity, T2DM, and GDM, and how these factors may contribute to adverse developmental programming of the offspring.

Definition and composition of the human microbiome

The human microbiome is made up of a microbial community comprising of over 100 trillion cells and associated genes, thus outnumbering human cells and genes by a factor of 10 and 27, respectively (Lloyd-Price et al. 2016). These microorganisms reside internally in multiple organs and externally on the skin as described in the HMP (Human Microbiome Project 2012). With the advent of next-generation sequencing, genomic information of each microbe can now be sequenced and thus allowing for a better understanding of the human microbiome (Cho & Blaser 2012). Furthermore, advances in the genomic and bioinformatic analysis have expanded knowledge of the microbial signature of each organ and how it interacts with physiology, which has improved the current understanding of how these microbes influence health outcomes.

The human microbiome includes bacteria, archaea, fungi, protists, and viruses (Lloyd-Price et al. 2016). These microbes are either commensal or have mutualistic relationships with the host (Lloyd-Price et al. 2016). However, on occasion, these nonpathogenic microorganisms can adversely alter human physiology by the production of certain metabolites (Kim 2018). The microbial community is variable within the human body. For instance, the common phyla that colonize the human gut include Firmicutes, Bacteroidetes, Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria (Arunugam et al. 2011). The oral cavity also harbors a diverse population of microorganisms, which is dominated by Streptococcus (Human Microbiome Project 2012). Skin is primarily colonized by Corynebacterium, Propionibacterium, and Staphylococcus (Grice et al. 2009). Similarly, a healthy vagina also has a microbial population dominated by Lactobacillus (Ravel et al. 2011, DiGiulio et al. 2015). The vaginal microbiota is noted to have an individual variation based on race and ethnicity and is further altered during pregnancy, as discussed subsequently (Ravel et al. 2011, DiGiulio et al. 2015).

Maternal microbiome changes during pregnancy

A woman’s normal microbiome undergoes significant changes during pregnancy, specifically in the gut, vaginal, and oral microbiomes (Nuriel-Ohayon et al. 2016) as summarized in Table 1.

Gut microbiome

The gut microbiota composition undergoes alterations during different stages of pregnancy. In the first trimester (T1), the gut microbiota mirrors the external environment and is similar to that of healthy, non-pregnant women analyzed in the HMP (Koren et al. 2012). However, as the pregnancy advances into the third trimester (T3), a decrease in microbial diversity is observed with a greater abundance of the pro-inflammatory phyla Proteobacteria and Actinobacteria (Koren et al. 2012). This change contributes to a decrease in intra-individual diversity, also referred to as alpha diversity. However, there is also a loss of different bacterial species in different women contributing to an increase in inter-individual diversity, also referred to as beta diversity (Koren et al. 2012). A decrease in anti-inflammatory phylum Faecalibacterium, a butyrate-producing bacterium typically depleted in patients with metabolic syndrome (Haro et al. 2016) and inflammatory bowel disease (Sokol et al. 2008), has also been observed during pregnancy.

In an attempt to better understand the role of T3 gut microbiota, one murine study transferred T1 and T3 gut microbiota samples from pregnant women to germ-free (GF) mice to observe their physiologic effects. After transplantation, the GF mice with T3 gut microbiota gained more weight, developed insulin resistance, and had a greater inflammatory response compared to the GF mice with T1 gut microbiota transplant (Koren et al. 2012). These changes resemble the changes seen in pregnant women across gestation and suggest that in pregnancy, the gut microbiota significantly contributes to the metabolic changes observed in the mother. The implications of such changes are yet to be determined.

Vaginal microbiome

In comparison to the gut microbiota, the vaginal microbiota remains relatively stable over the gestational period with an overall decrease in microbial diversity and richness. In comparison to healthy non-pregnant women, healthy pregnant women were found to have
Table 1  Maternal microbial alterations in healthy and disease states during pregnancy.

<table>
<thead>
<tr>
<th>Organ/pregnancy state</th>
<th>Key bacteria</th>
<th>Possible mechanisms</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut</td>
<td>Mirrors healthy, non-pregnant women</td>
<td>Decreased microbial diversity; increased pro-inflammatory Proteobacteria and Actinobacteria; decreased anti-inflammatory Faecalibacterium</td>
<td>Koren et al. (2012)</td>
</tr>
<tr>
<td>Healthy pregnancy</td>
<td></td>
<td>Alteration in hormones in overweight and obese pregnancies, including insulin, gastric inhibitory polypeptide, and adipokines, correlate with changes in bacterial composition.</td>
<td>Cani et al. (2007), Collado et al. (2008, 2010)</td>
</tr>
<tr>
<td>T1</td>
<td></td>
<td>Decreased alpha diversity. Changes in microbial composition of family Christensenellaceae and genera Lachnospira, Parabacteroides, Blautia, and Blautia. Increased Staphylococcus, Enterobacteriaceae, and Escherichia coli. Decreased Blautia.</td>
<td>Stanislawski et al. (2017)</td>
</tr>
<tr>
<td>T3</td>
<td></td>
<td>Decreased microbial diversity; increased pro-inflammatory Proteobacteria and Actinobacteria; decreased anti-inflammatory Faecalibacterium</td>
<td>Santacruz et al. (2010)</td>
</tr>
<tr>
<td>Overweight and obese pregnancies</td>
<td>Increased Staphylococcus and Clostridium histolyticum. Decreased Bifidobacteria</td>
<td></td>
<td>Collado et al. (2010), Santacruz et al. (2010), Gomez-Arango et al. (2016)</td>
</tr>
<tr>
<td>GDM</td>
<td>Decreased Faecalibacterium/Fusobacterium ratios. Increased Blautia and Collinsella, which are noted to activate a pro-inflammatory response and decrease liver glycogenesis. Increased Ruminococcaceae in early pregnancy.</td>
<td></td>
<td>Mokkala et al. (2017)</td>
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<td></td>
<td>Mokkala et al. (2017)</td>
</tr>
<tr>
<td>Vaginal</td>
<td>Lower microbial diversity and greater domination of Lactobacillus, particularly of Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, and Lactobacillus jensenii. Increased estrogen in pregnancy contributes to increased vaginal mucosa thickness and more deposition of glycogen for Lactobacillus to utilize.</td>
<td></td>
<td>Verstraeten et al. (2009), Spurbeck and Arvidson (2010), Aagaard et al. (2012), MacIntyre et al. (2015), Petrova et al. (2015), Freitas et al. (2017)</td>
</tr>
<tr>
<td>Healthy pregnancy</td>
<td></td>
<td>Proposed that changes to estrogen and progesterone in pregnancy contribute to oral microbiome.</td>
<td>Fujiwara et al. (2017)</td>
</tr>
<tr>
<td>Oral</td>
<td>Increased Prevotella/Aerococcus ratios.</td>
<td></td>
<td>Wang et al. (2018)</td>
</tr>
<tr>
<td>Healthy pregnancy</td>
<td>Increased microbial count; Increased periodontal pathogenic bacteria, Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans; Increased Candida.</td>
<td></td>
<td>Wang et al. (2018)</td>
</tr>
<tr>
<td>GDM</td>
<td>Increased Neisseria/Leptotrichia ratios.</td>
<td></td>
<td>Wang et al. (2018)</td>
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<tr>
<td>Placental</td>
<td></td>
<td></td>
<td>Aagaard et al. (2014), Lauder et al. (2016), de Goffau et al. (2019), Olomu et al. (2020), Sterpu et al. (2021)</td>
</tr>
<tr>
<td>Healthy pregnancy</td>
<td>Studies have had conflicting findings regarding the presence of a placental microbiome.</td>
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T1, first trimester; T3, third trimester.

lower microbial diversity and greater domination of the Lactobacillus species (Freitas et al. 2017). While there is no consensus on a single predominant Lactobacillus species in pregnancy, the most frequently isolated are Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, and Lactobacillus jensenii (Verstraeten et al. 2009, MacIntyre et al. 2015). Although the explanation for these changes is not well understood, a relationship between sex hormone levels and vaginal microbiota has been proposed (Freitas et al. 2017). As estrogen levels rise in pregnancy, there is an increase in thickness of the vaginal mucosa and more deposition of glycogen. Lactobacillus utilizes this glycogen for the production of lactic acid and thus, more glycogen in pregnancy may contribute to the greater dominance of Lactobacillus and lower overall microbial diversity in this group (Freitas et al. 2017). Lactobacillus dominance may also be explained by its bactericidal activity against other species (Spurbeck & Arvidson 2010) and its protection against infection by the maintenance of a pH < 4.5 and secretion of protective metabolites (Aagaard et al. 2012, Petrova et al. 2015). These findings have remained consistent among different studies despite differences in the cohort studied (Freitas et al. 2017).

**Oral microbiome**

As for the oral microbiota in pregnancy, the total viable microbial count is higher in all stages of pregnancy, especially in early pregnancy, compared to that of non-pregnant women (Fujiwara et al. 2017). Levels of periodontal pathogenic bacteria, such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans, are also significantly higher in the early and middle...
stages of pregnancy compared to non-pregnant women (Fujiwara et al. 2017). Levels of Candida, a common etiopathological agent in vaginitis, were also higher in the middle and late stages of pregnancy compared to non-pregnant women (Fujiwara et al. 2017). While some studies suggest that changes in estrogen and progesterone levels in pregnancy affect the oral microbiota (Fujiwara et al. 2017), these effects are not yet well understood. This is an area of interest for future research as a correlation between oral bacterial infections and pregnancy complications, particularly maternal periodontal disease and preterm birth, has been observed (Odenbach and others 2006, Zi et al. 2014). Additionally, the placental microbiome is non-invasive, and future studies may reveal potential biomarkers that may aid in the early detection and prevention of these diseases, especially preterm delivery.

**Placental microbiome**

Until recently, the placenta was considered to be a sterile environment. However, recent research found that the placenta harbors its own unique microbiome (Aagaard et al. 2014). The study by Aagard et al. using a population-based cohort study of 320 placental tissue samples was the first to record the existence of a unique placental microbiome. Using DNA sequencing techniques and metagenomic analyses, researchers found low-abundance, nonpathogenic commensal organisms, including Firmicutes, Tenericutes, Proteobacteria, Bacteroidetes, and Fusobacteria in the placenta (Aagaard et al. 2014). In fact, the placental microbiota was found to be similar to that of the normal human oral microbiota (Aagaard et al. 2014). One limitation of the study is that the placental microbiome was compared with results from the HMP and did not include positive or negative controls. Additionally, other studies have reported associations between the composition of the placental microbiota and pregnancy complications, such as preterm birth and periodontal disease (Zheng et al. 2015, Corbella and others 2016). However, while some studies do point to the evidence of a distinct placental microbiome, several subsequent studies have failed to replicate these findings (Lauder et al. 2016, de Goffau et al. 2019, Olomu et al. 2020, Sterpu and others 2021).

The low bacterial biomass in placental samples increases the risk that some or all of the bacterial DNA may have been derived from contamination, DNA isolation kits, or other commercial reagents (Lauder et al. 2016, de Goffau et al. 2019). A study by de Goffau et al. also found that vaginal organisms, such as Lactobacilli, were found in higher abundance in vaginally delivered placentas than intrapartum or pre-labor C-section placentas, thus suggesting that placental tissue samples were contaminated during the process of labor and delivery itself, even when dissected from within the placenta (de Goffau et al. 2019). Furthermore, a study by Lauder et al. compared the oral, vaginal, and placental samples of healthy deliveries to a matched set of contamination controls to determine the presence of a unique placental microbiome. While both oral and vaginal samples yielded a distinct, characteristic DNA composition, the placental sample results could not be differentiated from the contamination control. Two different methods of DNA purification yielded the same overall result, further disputing evidence for a placental microbiome (Lauder et al. 2016). Given the contrasting results from studies of the placental microbiome, further studies that incorporate adequate positive and negative controls are needed to define the placental microbiome, if one indeed exists.

**S. Han, C. Collado and others (2021)**

Altering the maternal microbiome in disease states

**Obesity**

Maternal pathological states can impact microbial diversity and composition (Shreiner and others 2015). One such example is maternal obesity as outlined in Table 1. Animal models of diet-induced obesity during pregnancy have shown alterations in gut microbiota when compared to normal-diet pregnant cohorts (Gohir and others 2015). Similarly, human studies have also suggested that alterations in microbiota are related to abnormal weight gain during pregnancy. In one study, overweight pregnant women had significantly higher levels of Bacteroides and Staphylococcus in their gut microbiota as compared to normal-weight pregnant women (Collado and others 2010). Pre-pregnancy obesity and excessive gestational weight gain were also associated with decreased alpha diversity and taxonomic differences in the family Christensenellaceae and genera Lachnospira, Parabacteroides, Bifidobacterium, and Blautia (Stanislawska and others 2017). Clostridium histolyticum, analyzed as a representative of the Firmicutes phyla, was also higher in obese pregnant women further suggesting a link between obesity and gut microbiota (Collado and others 2010). Specific metabolic hormones, such as insulin, gastric inhibitory polypeptide, and adipokines were also found to be correlated with changes in bacterial composition in overweight and obese pregnant women. This relationship further supports the connection between gut microbiota and metabolic hormones in pregnancy (Gomez-Arango and others 2016).

In the aforementioned study by Collado and others, Bifidobacterium levels were higher in normal-weight women than in overweight women throughout pregnancy (Collado and others 2010). Bifidobacteria has previously been found to be positively correlated with the normalization of inflammatory status, improved glucose tolerance, and improved glucose-induced insulin secretion (Cani and others 2007). Thus, the low levels of Bifidobacteria found in overweight pregnant women are suggestive of
increased inflammatory processes and further support a link between gut microbiota and pathological states (Collado et al. 2008). Similarly, a study by Santacruz et al. found decreased levels of *Butidobacterium* in overweight pregnant women and increased levels of *Staphylococcus*, *Enterobacteriaceae*, and *Escherichia coli* (Santacruz et al. 2010). In contrast, Santacruz et al. also noted decreased levels of *Bacteroides* in overweight pregnant women as opposed to the increased levels found in the study by Collado et al., highlighting some of the discrepancies in the literature relating maternal obesity to the gut microbiome. Additional studies are needed to address microbiota alterations in maternal obesity and their implication on maternal–fetal health.

**Gestational diabetes mellitus**

Alterations to the maternal microbiome are also seen in GDM pregnancies as summarized in Table 1. In one study by Wang et al., the microbiome of women with GDM was altered at multiple body sites, including the oral cavity, vagina, and gut (Wang et al. 2018). Both oral *Neisseria/Leptotrichia* ratios and vaginal *Prevotella/Aerococcus* ratios were positively correlated to hyperglycemia, while intestinal *Faecalibacterium/Fusobacterium* ratios were negatively correlated with hyperglycemia. The meconium microbiota of the neonates was also altered in the offspring of women with GDM (Wang et al. 2018). These correlations highlight the impact of GDM and its associated hyperglycemic state on the maternal microbiota composition across body sites.

In another study, GDM diagnosed in T3 of pregnancy was associated with gut dysbiosis as compared to normoglycemic pregnant women (Crusell et al. 2018). Numerous changes to the gut microbial composition were found. Some of the changes were associated with lower insulin sensitivity while others were associated with higher plasma glucose concentration. Furthermore, some elements of gut dysbiosis noted in women with GDM were present 8 months after pregnancy. There was an increased abundance of *Blautia* in women with GDM, which aligns with previous findings of increased *Blautia* abundance in glucose-intolerant individuals (Egshaty et al. 2016) and high BMI individuals with an unhealthy metabolic state (Zeng et al. 2019). However, the role of *Blautia* is controversial as some studies report a direct association between *Blautia* and hyperglycemia, while others report that this is indicative of a healthy gut (Benítez-Páez et al. 2020). While some studies attribute this difference to strain-dependent effects on metabolism (Ferrocino et al. 2018), additional studies are needed to elucidate the role of *Blautia* in the pathogenesis of GDM.

In the study by Crusell et al. mentioned previously, *Collinsella* was also increased in the gut microbiota of postpartum women with GDM in a previous pregnancy (Crusell et al. 2018). *Collinsella* affects host metabolism by decreasing liver glycogenesis and activating a pro-inflammatory response (Ferrocino et al. 2018). *Collinsella* has also previously been associated with increased fasting levels of insulin (Zhang et al. 2013) and significantly increased in non-pregnant individuals with T2DM (Lambeth et al. 2015), which supports the finding that gut dysbiosis in GDM resembles changes seen in non-pregnant individuals with T2DM and associated metabolic traits. A study by Mokkala et al. also found a statistically significant difference in the relative abundance of *Ruminococcaceae* in early pregnancy between women who developed GDM and women who did not. Even after adjustments for potential confounding factors, such as pre-pregnancy BMI, diet, and family history of T2DM or metabolic syndrome, there was still a significant association, thus demonstrating a connection between changes in microbial composition and GDM (Mokkala et al. 2017). Lastly, *Faecalibacterium*, an anti-inflammatory commensal bacterium that is highly associated with fasting blood glucose (Ferrocino et al. 2018), was also found to be depleted in both T3 and postpartum women with GDM (Crusell et al. 2018).

While these studies do not reflect causal relationships, they do provide additional evidence toward the understanding that disease states are associated with altered diversity and composition of the host microbiome outside of the normal scope of change. The extent to which such changes in microbiome composition alter maternal physiological states and impact fetal health is an area of interest for future research.

**Maternal–fetal transmission of microbiome**

Associations between disease states and microbial dysbiosis are not only limited to the mother but also can extend to their offspring. Furthermore, an adverse *in-utero* environment during the critical time of organogenesis can result in detrimental long-term health outcomes in offspring (Barker et al. 1998, Osmond & Barker 2000). In this regard, *in-utero* programming via maternal microbiome has been a topic of recent research (Tamburini et al. 2016, Jasiarević & Bale 2019). Although exact mechanisms underlying *in-utero* fetal programming via gestational microbiome are still under investigation, outlined in Table 2 and detailed below are ways in which maternal microbiome contributes to fetal and neonatal microbial colonization and offspring health.

**Origins of the neonatal microbiome**

Recent studies have highlighted the existence of a neonatal microbiome. From the time of birth, the neonatal gut specifically is colonized with increasing numbers of *Bifidobacterium*, Bacteroidetes, and Firmicutes (Tanaka & Nakayama 2017), and over time, it evolves into a more diverse adult microbiome. The understanding for years was that the gut was sterile at
birth. Escherich found that pure meconium contained no trace of microbial elements but had been colonized by a rich microbial flora by the eighth day of life (Escherich 1988, Ferretti et al. 2018). However, over the years, subsequent studies have demonstrated the presence of bacteria in meconium samples, challenging the theory that the gut was sterile at birth (Jimenez et al. 2008). One of the most compelling studies demonstrated in-utero transmission of the microbiome utilizing a murine model. In this study, Enterococcus faecium strain administered orally to pregnant mice was not observed in the meconium of their pups, whereas it was not detected in the non-inoculated group (Jimenez et al. 2008). Other studies have demonstrated colonization of microbes in the amniotic fluid and umbilical cord blood (Jimenez et al. 2005, DiGiulio 2012, D’Argenio 2018). Moreover, studies have also demonstrated similarities between microbes in the meconium and those found in the amniotic fluid (Ardissone et al. 2014) and between the maternal gut and umbilical cord blood (Jimenez et al. 2005). Taken together, these studies suggest that the neonatal microbiome is influenced by the maternal microbiome, even in healthy pregnancies.

**Proposed mechanism of developmental programming: Delivery mode**

In addition to the transmission of the microbiome within the womb, the birthing process has also been associated with alterations to the neonatal microbiome. For example, infants born via vaginal delivery have been shown to have a higher number of bacterial taxa identified than infants born via C-section (Lif Holgerson et al. 2011). Studies also demonstrate that infants born vaginally have microbial colonies in the skin, gut, oral cavity, and nasopharynx, which more closely resemble the maternal vaginal microbiome, whereas infants born via C-section have microbial colonies more similar to the maternal skin microbiome (Penders et al. 2006, Dominguez-Bello et al. 2010, Backhed et al. 2015). Specifically, Dominguez-Bello et al. found infants born vaginally had a gut microbiota dominated by Lactobacillus, Prevotella, or Sneathia, whereas infants born via C-section had microbial colonies in the skin, oral cavity, nasopharynx, and meconium dominated by Staphylococcus, Corynebacterium, and Propionibacterium. Penders et al. also found that infants born via C-section had higher numbers of C. difficile and lower numbers of Bifidobacterium and Bacteroides (Penders et al. 2006). Likewise, Biasucci et al. demonstrated differences in fecal samples 3 days after birth between infants born vaginally and via C-section. Bifidobacterium numbers were also significantly lower in infants born via C-section, with an overall decrease in gut diversity (Biasucci et al. 2008). Interestingly, low levels of Bifidobacterium in the infant gut microbiome have been associated with obesity risk, and it has been proposed that alterations to the microbiome may explain the association between C-section and development of obesity in offspring (Koleva et al. 2015, Kuhle et al. 2015). Overall, these studies underscore the importance of considering delivery mode when evaluating offspring microbiome.

**Proposed mechanism of developmental programming: Transfer of bacterial metabolites**

While there is strong evidence for the role of delivery mode in the composition of offspring microbiome, associations exist between maternal obesity, GDM, and offspring microbiome after controlling for delivery mode (Collado et al. 2010, Cerdo et al. 2018). In this regard, other mechanistic processes such as the transfer of microbial metabolites may be involved in linking maternal health to the offspring microbiome. Furthermore, maternal dysbiosis can lead to protein degradation and alteration in amino acid (AA) metabolism, a precursor for the production of metabolic end products.
such as short-chain fatty acids (SCFAs). SCFAs are gut microbiome byproducts produced through anaerobic fermentation and these include acetate, propionate, and butyrate. SCFAs are involved in epigenetic modification, immune regulation, and metabolism and are used for energy, lipogenesis, gluconeogenesis, and regulation of cholesterol synthesis (Soderborg et al. 2016, Stiensma & Turvey 2017, Hu et al. 2019, Nyangahu & Jaspan 2019).

Thus, it has been suggested that SCFAs may contribute to the pathogenesis of metabolic diseases, including insulin resistance and T2DM (Canfora et al. 2019).

In a study by Zheng et al., decreased relative abundance of butyrate-producing bacterium Coprococcus and lactate-producing bacterium Streptococcus that known to contain multiple SCFA-producing species was found in women with GDM. This suggests that decreased levels of SCFA-producing microbes in gut microbiota may be related to the development of glucose intolerance (Zheng et al. 2020). Of note, the presence of SCFA in meconium has been studied, and neonates born to mothers with T2DM had significantly lower levels of propionic acid in their meconium compared to mothers with normal pancreatic function or mothers with GDM (Stinson 2019). Similarly, Priyadarshini et al. noted that serum acetate levels were associated with maternal weight gain and adiponectin levels, while serum propionate was negatively associated with maternal leptin and neonatal length and body weight (Priyadarshini et al. 2014). While this study was limited to a small cohort, it highlights the role of SCFAs in maternal weight gain, hormone levels, and neonatal growth parameters. Furthermore, in a rodent model, Kimura et al. demonstrated the transfer of SCFA propionate from the colonic lumen of pregnant mice to embryos via maternal liver and bloodstream and the modulation of energy metabolism in the offspring (Kimura et al. 2020). They found that treatment of GF or low-fiber diet mice with propionate rendered adult offspring resistant to obesity.

Apart from SCFA, other AA metabolites have been implicated in pathological conditions. For example, specific AA have been studied in uterine secretions (e.g. arginine, leucine, proline, and glutamine) and allantoic fluid (e.g. aspartate, glutamate, glutamine, leucine, proline, and taurine) (Wu 2009, Dai et al. 2015), and differences in the metabolism of certain AA have been associated with obesity and T2DM (Neis et al. 2015). In a study by Mueller et al., neonates born vaginally to overweight or obese mothers had higher bacterial gene content related to amino acids (alanine, aspartate, glutamate, and histidine) but lower gene content related to the metabolism of other amino acids (lysine, tryptophan, valine, leucine, isoleucine) compared to the stool of neonates born to normal-weight mothers (Mueller et al. 2016). Alterations to concentrations of AA metabolites in the uterus are thought to impact cellular signaling pathways and nutrient transport and metabolism in offspring (Kim et al. 2011).

Further studies are needed to elucidate the mechanisms by which maternal dysbiosis influences fetal development and offspring health outcomes, and such studies should include AA and SCFA to further clarify their role in this process.

**Maternal–fetal transmission of microbiome in maternal disease states**

*Influence of maternal GDM on neonatal microbiome*

As some research has demonstrated maternal influences on the neonatal microbiome, researchers have wondered whether GDM or pre-pregnancy T2DM affects the infant microbiome. There is growing evidence that GDM and gestational hyperglycemia are associated with an increased risk for both short- and long-term adverse outcomes in the offspring (Dodd et al. 2007, HAPO Study Cooperative Research Group et al. 2008). Furthermore, offspring born to mothers with GDM have nearly double the risk of developing childhood obesity and/or metabolic syndrome compared to offspring born to non-diabetic mothers (Vohr & Boney 2008). With the growing literature demonstrating microbial sensitivities to the in-utero environment, there is a reason to believe that hyperglycemia may alter the developing neonatal microbiome (Mueller et al. 2015) and these alterations are listed in Table 2, which in turn may contribute to adverse programming of the fetal organs leading to postnatal short- and long-term complications. In this regard, levels of Bacteroides, Parabacteroides, and Lachnospiraceae were noted to be higher in meconium samples of offspring born to mothers with pre-pregnancy T2DM compared to offspring of mothers without T2DM (Hu et al. 2013). These bacteria have been shown to be prevalent in populations of adults with diabetes as well (Wu et al. 2010, Wang et al. 2018). Interestingly, there was an increase in alpha diversity most pronounced in those with pre-pregnancy T2DM as compared to GDM or non-GDM groups, leading Hu et al. to suggest that pre-pregnancy T2DM may promote microbial transmissibility (Hu et al. 2013). Of note, there were no differences observed based on the delivery mode in this particular study.

Another study examining the microbiome of infants born via C-section demonstrated that infants born to mothers with GDM had altered microbiota as compared to healthy pregnancies, such as higher prevalence of Prevotella, Streptococcus, Bacteroides, and Lactobacillus. Furthermore, positive correlations between certain maternal and neonatal microbiota and abnormal oral glucose tolerance tests were demonstrated (Wang et al. 2018). Moreover, Su et al. demonstrated that meconium from infants born to mothers with GDM was found to have lower microbial diversity and a difference in abundance of species as compared to infants of healthy mothers (Su et al. 2015).
et al. 2018). Infants born to mothers with GDM had increased phyla Proteobacteria and Actinobacteria and decreased Bacteroidetes. Of note, Proteobacteria is typically increased in diabetes and obesity, whereas Bacteroidetes is decreased (Ley et al. 2005, Turnbaugh et al. 2009). Furthermore, several unique gut microbiota of the phyla Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Chloroflexi, Acidobacteria, and Plantomycetes found in offspring of healthy pregnancies were absent in offspring of GDM pregnancies. At the genus level, there were lower levels of Prevotella and Lactobacillus in newborns of mothers with GDM (Su et al. 2018). More recently, gut microbiota of 2-week-old neonates born to mothers with GDM had a difference in the abundance of taxa, several of which are important for establishing neonatal immunity (Soderborg et al. 2020).

Additionally, Hasan et al. demonstrated that the genus Anaerotruncus, shown to have an association with glucose intolerance and gut permeability, was more abundant in the gut microbiota of offspring born to mothers with GDM (Hasan et al. 2018). This study also demonstrated a greater abundance of the genus Victivallis and the family Victivallaceae. These studies suggest that infants born to mothers with pre-pregnancy T2DM or GDM may experience alterations to their gut microbiome, which may lead to lifelong health implications. Future longitudinal studies are needed to further characterize the role of vertical transmission of the maternal microbiome in maternal disease states and the relationship to offspring health outcomes.

**Influence of maternal obesity on neonatal microbiome**

Maternal overweight or obesity has been shown to influence offspring microbiome and health as outlined in Table 2. In the Canadian Healthy Infant Longitudinal Development birth cohort, infants born vaginally to overweight or obese mothers were three times more likely to become overweight at age one, while infants born via C-section to overweight mothers had a five-fold increased risk of becoming overweight at age one (Tun et al. 2018). In their cohort, Firmicutes richness, specifically, higher abundance of the family Lachnospiraceae mediated the intergenerational transmission of overweight status. Birth mode was also thought to play a role as the effect was more pronounced for infants delivered via C-section (Tun et al. 2018). Interestingly, studies have demonstrated that Firmicutes promote adiposity, inflammation in body fat, and development of diabetes in mice (Cho & Blaser 2012, Ravussin et al. 2012).

Firmicutes have been shown to be significantly enriched in infants born to normal-weight mothers whereas Bacteroides was significantly higher in infants born to obese mothers (Cerdo et al. 2018). Interestingly, the authors did not find similar associations at 9 and 18 months of age. In another study, Collado et al. demonstrated that fecal Bacteroides, Staphylococcus, and Clostridium concentrations were significantly higher during the first 6 months of life in infants born to overweight mothers, whereas Bifidobacterium concentrations were lower (Collado et al. 2010). Staphylococcus has been proposed as having a role in weight gain as studies have demonstrated that pre-pregnancy weight and BMI are associated with higher levels of Staphylococcus (Collado et al. 2008). Furthermore, this study demonstrated a high prevalence of Akkermansia muciniphila in infants born to mothers with obesity and those with excessive weight gain during pregnancy. A. muciniphila is thought to play a role in the pathogenesis of inflammatory diseases (Campieri & Gionchetti 2001, Shih & Targan 2008). More recently, higher levels of Bacteroides fragilis, E. coli, and Veillonella dispar were associated with maternal obesity in vaginally delivered infants, while no associations were demonstrated in infants delivered via C-section (Singh et al. 2020).

Studies have also pointed out that the mode of delivery may influence offspring microbial communities. For instance, Mueller et al. demonstrated higher levels of Bacteroides in infants born to obese or overweight mothers, though only in those born via vaginal delivery. Thus, it was suggested that the associations stemmed from vertical transmission during labor rather than gestation (Mueller et al. 2015). On the other hand, the aforementioned studies by Collado et al. and Cerdo et al. demonstrated that maternal weight influences neonatal microbiome even when controlling for the mode of delivery (Collado et al. 2010, Cerdo et al. 2018).

While these studies have demonstrated offspring microbiome associations with gestational environment and delivery mode, antibiotic use and breastfeeding also influence the developing infant gut microbiome, which is the focus of other reviews (Panmaraj et al. 2017, Lv et al. 2018, Calatayud et al. 2019). There is strong evidence that early exclusive breastfeeding can prevent diseases later in life such as asthma, celiac disease, and obesity (Armstrong et al. 2002, Kull et al. 2010). Therefore, it is not surprising that early changes to the microbiome have been associated with adverse health outcomes later in life.

**Neonatal microbiome and future health**

**Influences of the neonatal microbiome on the development of atopy**

As noted previously, growing evidence suggests that disruptions to the microbiome early in life are linked to disease susceptibility later in life (Wilczynska et al. 2019). Animal studies have demonstrated increased allergic responses in GF mice that lack a microbiota (Herbst et al. 2011). Forsythe et al. showed that supplementing mice with pre-biotic fibers and probiotic bacteria could significantly reduce allergic inflammation in their offspring (Forsythe et al. 2016).
with Lactobacillus reuteri was protective against allergic airway responses (Forsythe et al. 2007). Specifically, the microflora hypothesis theorizes that the microbiota of individuals with allergies differs from those without and that differences are detectable prior to the onset of atopy (Shreiner et al. 2008). Furthermore, it suggests that the rise in incidence of allergic airway disease in industrialized countries may be due to altered microbial diversity secondary to antibiotic use (Noverr & Huffnagle 2005). In one small human study, meconium dominated by lactic acid bacteria was associated with respiratory problems in the infant (Gosalbes et al. 2013). Likewise, observed differences in the gut microbiome of infants at 1 month of age predicted the development of atopy at 2 years of age (Penders et al. 2007). Specifically, E. coli was associated with eczema and C. difficile was associated with eczema, wheezing, and allergic sensitization.

Moreover, mounting evidence suggests that there may be a critical window in early life in which disruptions to the microbiome lead to disease later in life (Stiensma & Turvey 2017). In humans, low gut microbial diversity evaluated at 1 week and 1 month of age was associated with asthma development at 7 years of age, though microbiome composition at 12 months of age was not (Abrahamsson et al. 2014). Arrieta et al. observed transient gut microbial dysbiosis during the first 100 days of life in a group of infants at risk of asthma, with decreases in abundance of Lachnospira, Veillonella, Faecalibacterium, and Rothia (Arrieta et al. 2015). These changes were less apparent by 1 year of age. They then inoculated GF mice with these four bacterial taxa and observed reduced airway inflammation (Arrieta et al. 2015), lending support to the role of these taxa in protection from the development of asthma. Future studies should assess the microbial community in samples taken across multiple time points to further understand the timing of these associations.

Interestingly, intake of SCFA metabolite acetate by pregnant mice attenuated allergic airway disease in their offspring (Thorburn et al. 2015) suggesting the contribution of maternal SCFA in the programming of atopy and reactive airway disease. Similarly, maternal GDM has also been associated with offspring development of atopic dermatitis and allergen sensitivity (Kumar et al. 2009), and as seen in atopic dermatitis, microbiota of infants born to women with GDM have been shown to have lower microbial diversity (Su et al. 2018). Bifidobacterium particularly has been reported in lower concentrations in allergic infants (Bjorksten et al. 2001), as well as in lower concentrations in infants born to obese mothers.

Influences of the neonatal microbiome on the development of obesity

In addition to associations between perturbations in the early microbiome and atopy, researchers have also observed similar findings with other diseases, such as celiac disease (Olivares et al. 2018), irritable bowel disease (O’Mahony et al. 2015), and obesity (Koleva et al. 2015). In a study of fecal samples from infants at 3, 26, and 52 weeks of age, high intestinal B. fragilis and low Staphylococcus concentrations between the ages of 3 weeks and 52 weeks were associated with a higher risk of obesity later in life (Vael et al. 2011). In line with these findings, a study of GF mice revealed weight gain and fat deposition after intestinal colonization by Bacteroides thetaiotaomicron (Backhed et al. 2004). Vael et al. proposed that Bacteroides are adaptive during the breastfeeding period, contributing to weight gain and energy storage. In addition, in the fecal microbiome samples of infants, Bifidobacterium was higher, and Staphylococcus was lower in children who remained normal weight at seven years of age vs those who became overweight (Kalliomaki et al. 2008). Thus, Kalliomaki et al. suggested that Bifidobacterium may contribute to low-grade inflammation and the onset of obesity, as studies have demonstrated links between inflammatory markers and Staphylococcus. In addition, a review by Kozyrskyj et al. found that higher Lactobacillus and lower Bacteroides within 3 months of birth predicts risk for infant and child overweight status (Kozyrskyj et al. 2016). Although there is growing evidence that dysbiosis is associated with the onset of disease, more research is needed to demonstrate causal relationships.

Conclusion

At present, studies show associations among maternal metabolic perturbation, maternal–fetal dysbiosis, and the development of disease in offspring. However, a few to date have conducted longitudinal studies demonstrating the specific impact of maternal–fetal microbial composition on health outcomes. Future studies should use serial samples from multiple microbiome sites in mothers, from pregnancy through parturition and from infants. Infant samples should also include multiple assessments over time as studies have demonstrated differences in the microbiome composition with age. Furthermore, studies should include high sample numbers of pregnant women and offspring from diverse racial backgrounds and geographical locations, including developed and underdeveloped countries to account for the Hygiene Hypothesis (Bendiks & Kopp 2013). Studies should control for antibiotic and medication use in pregnancy as well (Lv 2018). Social determinants such as diet, physical activity, and sleep may also be helpful to consider as these may impact maternal and neonatal microbiomes, as well as other environmental and

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genetic factors. Lastly, including mode of delivery and measures of microbial metabolites such as SCFA and AA in future studies may elucidate underlying mechanisms. Overall, further characterization of the maternal microbiome in disease states and its role in the developmental programming of offspring microbiome may contribute to strategies to improve maternal and offspring health.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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A K V conceived the review of literature. S H, C C E, N O, and A K V performed literature search. S H, C C E, N O, and A K V contributed to writing the manuscript.

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