Can we modulate the breastfed infant gut microbiota through maternal diet?

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One sentence summary: Maternal diet during the lactation period likely has direct and indirect impacts on the infant gut microbiome, via modulations to the milk microbiota and other milk components.

Abstract

Initial colonisation of the infant gut is robustly influenced by regular ingestion of human milk, a substance that contains microbes, microbial metabolites, immune proteins, and oligosaccharides. Numerous factors have been identified as potential determinants of the human milk and infant gut microbiota, including maternal diet; however, there is limited data on the influence of maternal diet during lactation on either of these. Here, we review the
processes thought to contribute to human milk and infant gut bacterial colonisation and provide a basis for considering the role of maternal dietary patterns during lactation in shaping infant gut microbial composition and function. Although only one observational study has directly investigated the influence of maternal diet during lactation on the infant gut microbiome, data from animal studies suggests that modulation of the maternal gut microbiota, via diet or probiotics, may influence the mammary or milk microbiota. Additionally, evidence from human studies suggests that the maternal diet during pregnancy may affect the gut microbiota of the breastfed infant. Together, there is a plausible hypothesis that maternal diet during lactation may influence the infant gut microbiota. If substantiated in further studies, this may present a potential window of opportunity for modulating the infant gut microbiome in early life.

Introduction

Early life is an important period during which the infant gut microbiome is established. There is increasing evidence that development of the gut microbiota during infancy and early childhood may play an important role in programming health and disease later in life (Tanaka and Nakayama 2017). In support of this, perturbations to the early-life gut microbiota have been associated with the development of chronic diseases, including allergy, asthma, and obesity in both child and adulthood (Gomez-Gallego 2016; Stiensma and Michels 2018; Stinson 2019a).

Mature human milk (HM) is a major driver of infant gut microbial development (Bäckhed 2015; Parigi 2015; Stewart 2018). Breastfeeding has been associated with protection against respiratory and gastrointestinal pathogens and reduces the risk of inflammatory disorders, including atopy, asthma, obesity, diabetes and inflammatory bowel disease (Gunderson 2015;
Kelishadi and Farajian 2014; Work Group on Breastfeeding 1997; Xu 2017). HM shapes the infant gut microbiome through transfer of microbes (Asnicar 2017; Duranti 2017; Jiménez 2015; Kordy 2020; Milani 2015; Ward 2013), as well as other bioactive molecules such as human milk oligosaccharides (HMOs) (Lewis 2015; Wang 2015), short chain fatty acids (SCFAs) (Hassiotou 2013; Jiang 2016; Lewis 2015; Mohanty 2016; Wang 2015), and antimicrobial peptides (AMPs) (Mohanty 2016). HM is thought to be colonised with microbes via a number of pathways, including maternal gut microbes via the entero- mammary pathway (Rodríguez 2014), maternal skin microbes from the breast/areola surface (Moossavi 2019; Murphy 2017), and infant oral microbes through retrograde flow during feeding (Ramsay 2004; Ramsay 2005). There may also be a resident mammary tissue microbiome prior to lactation (Chan 2016; Costantini 2018; Meng 2018; Urbaniak 2014; Urbaniak 2016).

The effect of diet on the gut microbiota of adults has been well-characterised (Fava 2012; Kovatcheva-Datchary 2015; Wu 2011b; Yatsunenko 2012). The adult gut microbiota can be modified by altering dietary intake, such as modulating the amount and type of food groups ingested (carbohydrates, proteins, and fat), or by consuming prebiotics (indigestible fibres that are selectively fermented by the gut microbiota) and/or probiotics (live microorganisms that confer health benefits to the host) (Houghton 2016). Importantly, the effects of diet on the gut microbiome can be rapid (David 2014). As such, modulations to the maternal diet during the lactation period may influence the HM microbiome, which in turn may shape the infant gut microbiome. If the diet of a lactating mother influences the infant gut microbiota during early life, then this may provide a potential opportunity to intervene during this critical period of development to optimise the infant microbiome and associated short and long-term health outcomes.
In this review, we will provide an overview of the interactions among the maternal gut, HM, and infant gut microbiomes. Given that, to date, only one study has investigated the impact of maternal diet during lactation on the infant gut microbiome, we will draw together evidence from animal studies, studies of the effect of the gestational diet on the infant gut microbiome, and studies of the effect of diet on the HM microbiome, to identify dietary factors that are likely to influence infant gut bacterial composition. Finally, we will provide novel insights into the potential use of maternal dietary interventions during lactation with the aim of modifying the infant gut microbiota for improved short/long-term health outcomes.

Method

A search was performed in PubMed, Google Scholar, ProQuest and One Search (English) with the following combinations of search terms: maternal diet, pregnancy; maternal diet, lactation; maternal diet, infant gut microbiome/microbiota; maternal diet, human/breast milk microbiome/microbiota; human/breast milk, maternal gut microbiome/microbiota; human/breast milk, infant gut microbiome; maternal gut microbiota; human/breast milk microbiota; infant gut microbiota. The search covers the period prior to December 2020.

Human milk as a link between the maternal and infant gut microbiomes

Human milk and its relationship to the infant gut microbiome

Initial colonisation of the intestinal tract is a complex process, involving microbe-host and microbe-microbe interactions. Ingestion of HM, either exclusively or partially, has been shown to be the most significant factor associated with infant gut bacterial composition and the only factor associated with the predicted function of the gut bacteria, with lipid and...
carbohydrate metabolism pathways significantly associated with breastfeeding (Bäckhed 2015; Stewart 2018). These relationships are thought to be due to vertical transmission of microbes from mother to infant via HM (Asnicar 2017; Duranti 2017; Jiménez 2015; Kordy 2020; Milani 2015; Ward 2013), as well as the transfer of non-microbial factors (Hassiotou 2013; Jiang 2016; Lewis 2015; Mohanty 2016; Wang 2015).

A number of studies have identified microbes that are shared between paired HM and infant gut samples, including bifidobacterial species and bacteriophages (Asnicar 2017; Duranti 2017; Jost 2014; Kordy 2020; Milani 2015; Ward 2013). The use of whole genome shotgun sequencing and single nucleotide variant analysis has allowed high resolution profiling of these sample types, providing strong evidence of strain sharing between HM and the infant gut. However, it should be noted that this does not necessarily demonstrate vertical transfer, as a shared environmental source may exist.

Non-microbial HM components, such as HMOs, also drive early infant gut colonisation patterns. HMOs are a family of complex glycans found in HM (Bode 2012) and have been associated with infant gut bacterial composition and function. This diverse group of glycans act as prebiotics, which are specifically metabolised by a small number of bacterial taxa, including Bifidobacterium spp. These bacteria exhibit altruistic cross-feeding behaviours by breaking down HMOs to make other carbohydrates available to other members of the gut microbiome (Asakuma 2011). This co-operative behaviour has a substantial influence on the composition of the infant gut microbiome (Turroni 2018). Of the seven most prevalent bifidobacterial species in HM, Bifidobacterium bifidum and Bifidobacterium breve have the highest enzymatic potential to metabolise HMOs (Lugli 2020). AMPs, such as lactoferrin and lysozyme, exert antimicrobial activity, and are important mediators of protection against gastrointestinal infections in breastfed infants (Wada and Lönnerdal 2014). Several studies have shown the ability of lactoferrin to influence infant gut composition (Mastromarino 2014;
Other HM components such as SCFAs may influence the infant gut microbiome; however, to date no study has investigated such effects. SCFAs, the end products of carbohydrate fermentation by gut bacteria, are present in HM (Gómez-Gallego 2018; Jiang 2016; Meng 2019; Precht and Molkentin 1999; Prentice 2019; Santillo 2018; Smilowitz 2013; Stinson 2020b; Wu 2016) and can act as substrates for metabolism by the infant gut microbiota. Therefore, levels of SCFAs in HM might influence the composition of the infant gut microbiome.

Intake of HM likely provides a selective advantage for certain infant gut taxa over others. For instance, infant inheritance of *Bacteroides uniformis* depends on whether the dominant maternal strain of this bacteria has genes for glycan utilisation (Yassour 2018). If these genes are lacking in the dominant maternal strain and present in the secondary strain, infants become colonised with the secondary strain. Glycan utilisation may assist in HM metabolism, which may explain the preferential colonisation with maternal secondary strains that have glycan utilisation genes (Yassour 2018). Associations between maternal HMO secretor status (i.e. active or inactive copy of the FUT2 gene) and the HM bacterial composition have been reported (Cabrera-Rubio 2019). Non-secretor mothers showed lower levels of *Streptococcus* spp., *Lactobacillus* spp., and *Enterococcus* spp. in colostrum, *Streptococcus* spp. and *Enterococcus* spp. in transitional milk, and *Streptococcus* spp. in mature milk compared to secretor mothers. Low prevalence of *Bifidobacterium* spp. in the milk of non-secretor mothers was also observed (Cabrera-Rubio 2019). The initial gut bacteria in breastfed infants preferentially utilise lactate and HMOs, while, after weaning to solid foods, the cessation of breastfeeding leads to changes in bacterial composition that promotes carbohydrates utilisation, vitamin synthesis and xenobiotic degradation (Stewart 2018; Vaishampayan 2010; Vallès 2014; Yatsunenko 2012). Collectively, the available evidence demonstrates the importance of HM in shaping early-life gut bacterial composition and function.
The maternal gut microbiome and its relationship with human milk

The origin of the HM microbiota remains unclear. Several studies have discussed different body sites as potential sources: the infant’s oral cavity, the maternal skin, and the maternal gut (Fernández 2013b; Martín 2004; Rodríguez 2014; Stinson 2020a). To date, however, conclusive evidence has not been produced to support any of these sources. It is likely that HM is continuously affected by exposure to different microbial populations related to the mother and her infant. Currently, the source of the HM microbiome for which the most robust evidence exists is the maternal gut (via entero-mammary pathway). Detection of obligate anaerobic bacterial species that are typical of the gut microbiota, such as Bacteroides spp. and Bifidobacterium spp., in HM strongly suggests an internal source of transfer (Jost 2014; Murphy 2017; Rodríguez 2014). A number of studies have also identified shared bacterial strains in the maternal gut and HM (Asnicar 2017; Duranti 2017; Kordy 2020; Milani 2015). Two studies, in which probiotics were orally administered to lactating women in an attempt to treat or prevent mastitis (Fernández 2016; Jiménez 2008), reported that the probiotic strains Lactobacillus salivarius CECT5713, Lactobacillus gasseri CECT5714 and L. salivarius PS2 were able to be recovered from 6/10 and 15/29 HM samples, respectively. The recovery of probiotic strains from HM is an outcome of a complex process that can be influenced by multiple factors. Host factors such as genetic and immunological factors, and the existing maternal gut microbiota, can play a role in this process (Zmora 2018). Sampling time may also contribute to the ability to detect the probiotic strain in HM, as transit time from ingestion to the breast will be influenced by bowel transit time and timing of transport from bowel to breast (which is yet to be studied). Further work is therefore required to quantify the ability of ingested probiotics to alter the HM microbiome.
These same strains have also been genetically labelled and orally administrated to pregnant and non-pregnant mice. These strains were detected in the mesenteric lymph node, mammary tissue, and milk in the pregnant group of mice, but not in the non-pregnant mice (de Andrés 2018). Their presence in the mesenteric lymph nodes of pregnant mice suggests an immunemediated and gestation-dependant route of transport.

Intestinal bacteria may also be transported to the lactating mammary gland by mononuclear cells, for example dendritic cells (Civardi 2013; Fernández 2013a; Perez 2007). Dendritic cells are able to transfer gut bacteria by traversing the intestinal epithelial cell tight junctions. In addition, these mononuclear cells are able to express tight-junction proteins, which maintain the integrity of the epithelial barrier (Rescigno 2001). Using a mouse model, Perez et al. investigated the process of intracellular bacterial translocation from the maternal gut to the mammary gland through the peripheral blood circulation (Perez 2007). A greater total number of bacteria were identified in the mesenteric lymph nodes of pregnant mice compared with non-pregnant mice. This may suggest increased transport of intestinal bacteria to the mammary gland through mononuclear cells during late pregnancy and lactation (Perez 2007). These results are consistent with those reported by Donnet-Hughes et al. who found that gut bacterial translocation was increased during pregnancy and lactation in mice and that there was an increase in bacteria loaded dendritic cells in lactating mammary glands (Donnet-Hughes 2010). Indeed, live bacteria can be harboured by intestinal dendritic cells in the mesenteric lymph nodes for up to 60 hours, and from here can spread to distal body sites via the lymphatic system (Macpherson and Uhr 2004). In addition to dendritic cells, other cell types, including CD18+ expressing phagocytes and macrophages have also been demonstrated to function in extra-intestinal transportation of non-invasive Salmonella spp. (Vazquez-Torres 1999). It is unclear how bacteria enter the mammary gland from the lymphatic system, though it is hypothesised that this process is also mediated by dendritic
cells in a similar fashion to gut bacterial translocation. It should also be noted that in human, during pregnancy and for 48 to 72 hours after delivery, the tight junctions that join the mammary epithelial cells are open (Neville 1991), potentially allowing greater importation of bacteria. It is unclear whether bacterial translocation to mammary tissue is a random or selective process.

Maternal gut bacteria may contribute to the composition of HM not only via direct transfer of bacteria, but also via their production of metabolites. Of specific interest are SCFAs, which are produced mainly in the colon then absorbed and distributed to the maternal circulation (Koh 2016). HM SCFAs are either transported from the maternal gut or synthesised locally by HM bacteria. The SCFAs acetate, butyrate, and formate have previously been detected in HM (Gómez-Gallego 2018; Jiang 2016; Meng 2019; Precht and Molkentin 1999; Prentice 2019; Santillo 2018; Smilowitz 2013; Wu 2016). Importantly, reduced levels of HM SCFAs have been associated with maternal atopy status (Stinson 2020b) and infant adiposity (Prentice 2019), suggesting that HM microbial metabolites likely play a role in developmental programming of non-communicable diseases. Maternal diet, particularly an increased intake of dietary fibre, can affect the levels of SCFAs that are synthesised in the gut and absorbed from the maternal circulation (Abell 2008; Brinkworth 2009; David 2014; De Filippo 2010; Schneider 2006; Tarini and Wolever 2010) and may therefore influence HM SCFA levels. Taken together, the evidence to date supports the maternal gut as a source of the HM microbiota and microbial metabolites; however, further research is required to better understand the mechanisms that allow the physiological translocation of bacteria from the maternal intestine to the HM and the total contribution of the maternal gut bacteria to the overall HM microbiome and metabolome.
Can the infant gut microbiota be modulated through maternal diet?

As the maternal gut microbiota are considered one of the sources of the HM microbiome, it may be expected that dietary modulation of the maternal gut microbiome could in turn influence the HM and thereby the infant gut microbiomes. Indeed, a small number of studies have provided preliminary evidence that the maternal diet during pregnancy has an impact on the infant gut microbiota (Chu 2016; Lundgren 2018; Ponzo 2019; Savage 2018). However, the effect of maternal diet during lactation on the infant gut microbiota has not yet been investigated.

Diet modulates the maternal gut microbiome

Gut bacterial communities are influenced by dietary patterns, both acutely, and in the long term (David 2014; De Filippis 2016; De Filippo 2010; Fava 2012; Graf 2015; Kovatcheva-Datchary 2015; Lin 2013; Wu 2011b; Yatsunenko 2012). While extensive studies have examined the influence of diet on the gut microbiome in adults, there is very limited evidence of such effects in pregnant and lactating individuals. This is particularly important as these are periods of extensive hormonal, metabolic, and immunological changes (Edwards 2017; Neuman 2015). Such changes are associated with modifications to the maternal gut microbiome, particularly in the final trimester of pregnancy (Chassaing and Gewirtz 2014; Koren 2012). There is evidence to suggest that the maternal gut microbiome can be modulated by diet during pregnancy (Mandal 2016; Röyttö 2017), however, these studies are limited and their results are conflicting.

Only three studies have examined the effect of maternal diet during pregnancy on maternal gut bacterial communities. One study showed no effect of consuming salmon (two 150 g pieces) per week from 20 weeks of pregnancy to delivery on maternal gut bacterial profiles (Urwin 2014), while two others showed compositional changes in gut bacterial profiles in
relation to maternal diet (Mandal 2016; Röytiö 2017). Röytiö et al. separated obese and overweight mothers into three groups based on their adherence to a reference dietary intake (according to Nordic Nutrition Recommendations); a low-fibre/high-fat group, a high-fibre/moderate-fat group, and a low-fibre/moderate-fat group. Compared with the low-fibre/high-fat group, those who consumed fat and fibre in accordance with the recommended reference values during pregnancy showed low-grade inflammation (measured as lower level of glycoprotein acetylation), a reduced abundance of gut Bacteroidetes, and a higher level of gut microbial richness (Röytiö 2017). This negative association between fibre intake and Bacteroidetes in gut microbiota has previously been reported in non-pregnant individuals (Lin 2013; Wu 2011a); however, some studies have reported a positive association (De Filippo 2010; Fava 2012). Since the participants in this study were all overweight or obese, these findings may not necessarily apply to women of normal weight, and therefore it is important to exercise caution when extrapolating these results to a broader context. Mandal et al. reported that high maternal consumption of fat-soluble vitamins during gestation, particularly vitamin D, was associated with decreased gut bacterial diversity (Mandal 2016). In this same study, increased maternal intake of vitamin D, cholesterol, monounsaturated fats and retinol was linked with an increased abundance of Proteobacteria. Conversely, maternal intake of vitamin E, saturated fat, and protein was associated with a decrease in the abundance of Proteobacteria (Mandal 2016). One limitation of this study is that maternal dietary intake during pregnancy was assessed during the second trimester, while characterisation of bacterial profiles in maternal stool samples was carried out four days post-delivery. This is significant as during the long period of time between these two time points, maternal hormones and dietary habits may have shifted considerably. In addition, the closure of the mammary epithelial tight junctions after birth (and before stool sample collection) may have affected transfer of microbes into the milk. Given that the effect of diet on the human
gut microbiota can be as rapid as one day (David 2014), the maternal gut microbiome may change significantly over this time. Further, given that pregnancy is associated with compositional changes in the gut microbiome (Chassaing and Gewirtz 2014; Koren 2012; Nuriel-Ohayon 2019), it is questionable whether a postnatal stool sample would be representative of the prenatal gut microbiome. The use of a single food frequency questionnaire administered in week 22 of pregnancy is another limitation of this study due to the potential for recall bias. Limited data on the effect of diet on the maternal gut microbiome during lactation exists, with one study suggesting that macronutrient and micronutrient intake is associated with the composition of the maternal gut microbiome during lactation (Carrothers 2015). Understanding the influence of maternal diet during pregnancy and lactation on the gut microbiome is important to improve dietary recommendations for pregnant and lactating women.

Maternal gut bacteria are transferred to the infant gut via HM

A small number of studies have provided evidence to support the theory of vertical transmission of maternal gut bacteria to the infant gut via HM (Asnicar 2017; Duranti 2017; Jost 2014; Kordy 2020; Milani 2015). These studies have reported shared bacterial strains in maternal faecal/milk samples and infant faecal samples using metagenomics and strain level analyses (Asnicar 2017; Duranti 2017; Kordy 2020; Milani 2015). Collectively, these studies have suggested that, among the many bacterial species found in infant stool and HM, only a limited number are mutually shared in mother-infant dyads (Table 1). In particular, *Bifidobacterium* species are consistently identified as shared features of the maternal gut, HM, and infant gut microbiomes. Although negative controls are not widely used in these studies and the sample sizes of the studies are small, the consistency of the results supports the hypothesis of strain sharing between mothers and infants via HM.
Experimental evidence also demonstrates the ability of maternally ingested bacteria to be transferred to breastfed infants. Maternal oral probiotic supplementation with *Lactobacillus rhamnosus* LGG from week 36 of pregnancy to three months postpartum resulted in infant gut colonisation with this strain (Dotterud 2015). However, this colonisation was transient and persisted for only 1-2 weeks following the cessation of maternal probiotic administration (Dotterud 2015). While a shared environmental source may explain the co-occurrence of this probiotic strain in maternal and infant stool samples, transfer via breastfeeding is a likely mechanism. Unfortunately, these authors did not collect HM samples, so we are only able to speculate as to the role of HM in this transfer. Regardless, this experimental evidence supports existing observational evidence (Table 1). Collectively, the evidence demonstrates the ability of maternally ingested bacteria to be transported to the infant gut via HM, providing a potential link between the maternal diet and the infant gut microbiome.

*Evidence that maternal diet influences the HM microbiome*

Maternal diet, which has been shown to modify gut microbiota (Mandal 2016; Röytiö 2017) and HM nutritional composition (Andreas 2015), may also modulate HM microbial composition (Figure 1). To date, five studies have investigated the influence of diet on the HM bacterial profile (Table 2). Williams et al. collected HM samples at multiple time-points (2, 5, and 10 days, and 1, 2, 3, 4, 5, and 6 months post-delivery) from healthy lactating women (n=21) (Williams 2017). Dietary intake of participants was assessed using a quantitative 24 hour dietary recall at the time of each sample collection. Several relationships were identified between dietary intake and HM bacterial profiles (Table 2). However, this study is limited by the use of mean values for HM microbiota and dietary intake variables over nine-time points (from day 2 to 6 months post-delivery), as acknowledged by the
authors themselves. This has significant implications for data interpretation as this period includes production of the three different types of HM (colostrum, transitional and mature HM) all of which have distinct bacterial communities (Boix-Amorós 2016; Cabrera-Rubio 2012; Khodayar-Pardo 2014). Both microbial composition and dietary patterns are also likely to vary across the first six months postpartum.

Padilha et al. characterised the bacterial communities in HM collected at day 30 (±4) postpartum from 94 healthy lactating women (Padilha 2019). Maternal diet during pregnancy was assessed using quantitative food frequency questionnaires at day 30 (±4) postpartum, and during the first month of lactation using two 24 hour dietary recalls at day 7 (±3) and day 30 (±4) after delivery. Many significant correlations between maternal nutrient intake and HM bacterial composition were identified. Most notably, the abundance of *Staphylococcus* spp. in HM was positively correlated with vitamin C intake during pregnancy.

LeMay-Nedjelski et al. reported associations between maternal intake of fat and fibre during lactation and the HM bacterial profile at three months postpartum in a cohort of 93 women with different metabolic conditions including, normoglycemic lactating women (n=56), and lactating women with gestational diabetes mellitus (n=21) or impaired glucose tolerance (n=16) (LeMay-Nedjelski 2020). Analysis of maternal dietary intake and HM microbiome was performed on the whole study population without subgroup analysis. Maternal intake of fibre from grains was associated with increased alpha and beta diversity, while polyunsaturated fatty acids (PUFAs) intake was positively associated only with alpha diversity of HM. Total fibre intake was associated with beta diversity of predicted bacterial functions (based on 16S rRNA gene-derived taxonomy). Several other associations were identified between the maternal dietary consumption of fat and fibres and bacterial genera (Table 2). However, it is important to consider that more than one-third of the women in this study were diagnosed with gestational diabetes mellitus or impaired glucose tolerance.
Metabolic diseases are associated with a distinct gut microbiota composition (Yang and Kweon 2016); therefore, the generalisability of these results is questionable. Regardless, the finding of a positive correlation between fibre intake and HM richness and diversity aligns well with similar findings in the gut microbiome (De Filippo 2010; Makki 2018; Schnorr 2014).

Babakobi et al. collected HM samples at three time-points (one week, one month, and three months postpartum) from healthy lactating women \( (n=22) \) (Babakobi 2020). Maternal dietary intake was assessed at 3 months postpartum using a food frequency questionnaire. At this time point participants were asked to recall both their current diet and their diet during gestation. Maternal diet during gestation and lactation were combined for the purposes of the analysis. In addition, 24 hour dietary recalls were performed one day prior to sample collection at each of the three time points to validate the food frequency questionnaires. The relative abundance of *Streptococcus* spp. was negatively associated with maternal intake of PUFAs, monounsaturated fatty acids, and folic acid at one month postpartum, and positively associated with vitamin B12 at three months postpartum. However, this study is limited by the inappropriate assessment of maternal dietary intake during lactation, which was combined with maternal dietary intake during pregnancy.

Recently, Cortes-Macías et al. characterised the bacterial profile of HM samples collected at day 11 \( (±4) \) postpartum from 120 healthy lactating women (Cortes-Macías 2020). Maternal gestational dietary intake was assessed using a food frequency questionnaire at day 11 \( (±4) \) postpartum. Women were grouped into two clusters based on their diet: cluster I (high intake of fibre, plant protein, and carbohydrates) and cluster II (high intake of animal protein and lipids). Numerous associations were reported between HM bacterial genera and dietary habits during pregnancy (Table 2). Inferred HM bacterial functions were also significantly associated with maternal diet. Interestingly, intrapartum antibiotic exposure and mode of
delivery were associated with HM bacterial composition in a diet-dependent manner. Milk from mothers in diet cluster II, those who delivered via caesarean section, and those exposed to intrapartum antibiotics had a significantly reduced relative abundance of *Lactobacillus* spp., *Bacteroides* spp., and *Sediminibacterium* spp. compared to the other groups. Overall, despite methodological limitations in the current literature, the evidence suggests that maternal diet is associated with HM bacterial composition.

**Evidence that maternal diet influences the infant gut microbiome**

Emerging observational evidence suggests that maternal diet influences the composition of the infant gut microbiota (Figure 1). Six studies have investigated the influence of gestational diet on the infant gut microbiota in humans (Table 3). In one study, the overall infant gut bacterial community structure at six weeks postpartum was found to differ according to maternal diet during weeks 24–28 of pregnancy (Lundgren 2018). In this cohort of 150 infants, only 70% were exclusively breastfed, while 26% were partially breastfed, and 4% were exclusively formula fed. Interestingly, the effect of maternal gestational diet on the infant gut microbiome was found to be delivery-mode dependant after adjusting for potential confounders such as feeding method, maternal BMI, parity, and batch (Lundgren 2018). Higher maternal fruit intake per day was associated with an increased likelihood of *Streptococcus* spp. and *Clostridium* spp. predominance in vaginally-delivered infants. In contrast, caesarean-delivered infants whose mothers consumed a higher amount of dairy products per day were more likely to fall into the high *Clostridium* spp. cluster (Lundgren 2018). The biological significance of this finding is unclear as *Streptococcus* spp. and *Clostridium* spp. are typical inhabitants of the gut microbiota (Jandhyala 2015).
Chu et al. compared the early gut microbiome of infants whose mothers consumed a high-fat gestational diet and those whose mothers consumed a low-fat diet in a cohort of 157 infants that were mixed fed (both HM and formula, except for two individuals who were exclusively formula fed). These authors reported that a maternal high-fat diet during pregnancy was associated with compositional changes in the neonatal gut microbiota at birth, that continued through until 4-6 weeks of age (Chu 2016). Specifically, a maternal high-fat diet was associated with an increased abundance of *Enterococcus* spp. and a reduced abundance of *Bacteroides* spp. in infant stool. Reduced gut *Bacteroides* spp. has been associated with a higher risk of obesity in adults (Armougom 2009; Furet 2010; Ley 2006; Turnbaugh 2006; Turnbaugh 2009); therefore, these infants may be at increased risk of developing obesity if these changes persist. However, other studies have reported contradictory results, whereby obese individuals had a higher abundance of *Bacteroides* spp. (Bervoets 2013; Schwiertz 2010; Zhang 2009), potentially due to differences between study populations, such as diet or geographical location (Dugas 2016). *Bacteroides* spp. are involved in fermenting HMOs to produce SCFAs, which influence many aspects of the immune system as well as glucose homeostasis and lipid metabolism (Kumari and Kozyrskyj 2017; Marcobal 2010; Salyers 1977; Thorburn 2014). Thus, depletion of *Bacteroides* spp. in the infant gut may impact early-life immune and metabolic development (Mazmanian 2005; Mazmanian 2008; Round and Mazmanian 2010; Troy and Kasper 2010).

Savage and colleagues collected infant stool samples (n=323) at 3-6 months postpartum. Most infants in this cohort were exclusively formula-fed (n=169), with only 95 exclusively breastfed. These authors reported that increased maternal consumption of vegetables and decreased consumption of deep-fried foods and processed meats during the second and third trimesters of pregnancy were negatively associated with *Bacteroides* spp. and *Clostridium* spp. and positively associated with *Lactobacillus* spp. in the infant gut (Savage 2018). After
adjusting for demographic variables, including mode of feeding, maternal diet was only positively associated with *Lactobacillus* spp. The increased abundance of *Lactobacillus* spp. in these infants may be beneficial due to the probiotic potential of members of this genus (Anderson 2010; Olivares 2006; van Baarlen 2013). It is important to note, however, that one or both parents included in the study were diagnosed with asthma or allergy, and thus the results cannot be extrapolated to a wider population. Further, by six months of age solid food was introduced to the diets of 42% of these infants. Solid food introduction can drastically change the infant gut microbiome (Bergström 2014; De Filippo 2010). Therefore, results from this study may not accurately reflect the effect of maternal diet during pregnancy on infant gut bacterial composition.

Ponzo et al. investigated associations between the dietary habits in mothers with gestational diabetes mellitus (GDM) (*n*=29) and the gut bacterial profiles of their infants during the first week of life (Ponzo 2019). Most of these infants received formula prior to sampling (*n*=19). Maternal dietary intake was assessed during gestational weeks 24-28 and again in the third trimester. Associations between maternal dietary variables and infant gut bacterial communities were reported, with a greater number of associations observed between the second trimester diet and the infant gut microbiome than the third trimester diet. In this study, maternal consumption of oligosaccharides was positively associated with levels of infant faecal *Ruminococcus* spp., while saturated fat consumption was negatively associated with infant faecal *Rikenellaceae* spp. and *Ruminococcus* spp. (Ponzo 2019).

García-Mantrana et al. investigated the influence of maternal diet during pregnancy on the infant gut microbiome. Characterisation of bacterial communities in meconium samples (*n*=86) showed their association with maternal diet during pregnancy. Increased maternal consumption of fibre and vegetable protein was negatively associated with infant gut
Bacteroidetes, while high maternal consumption of animal proteins during pregnancy was positively associated with infant gut Acinetobacteria (García-Mantrana 2020).

To date, only one study has included dietary assessment during lactation in their analysis of the infant gut microbiome (Babakobi 2020). Babakobi et al. collected infant stool samples at three time points (one week, one month, and three months postpartum) from healthy exclusively breastfed infants (n=22). Maternal dietary intake was assessed at 3 months postpartum, at which point participants were asked to recall both their current diet and their diet during gestation. Maternal diet during gestation and lactation were combined for the purposes of the analysis. No associations between maternal diet during gestation and lactation and infant gut bacterial composition were identified at any of the three time points of the study. However, the combined assessment of maternal dietary intake during both pregnancy and lactation in this study makes it difficult to draw conclusions on the specific effect of diet during lactation on the infant gut microbiome. Together, these studies highlight the influence of gestational diet on the infant gut microbiota; however, more appropriately designed studies are needed to better understand this relationship.

**Limitation in current observational studies**

All of the observational studies described above suffer from substantial limitations. Most notably in all six studies of the effect of maternal diet on the infant gut microbiome, a long period of time elapsed between assessment of maternal dietary habits (second or third trimester) and collection of infant stool samples 1 week, 6 weeks, 1 month, 3 months or 6 months (Babakobi 2020; Chu 2016; Lundgren 2018; Ponzo 2019; Savage 2018). This is an important consideration as dietary habits may change over such a long interval of time (from pregnancy to lactation) and diet can have a rapid effect on the gut microbiota (David 2014). In addition, the use of retrospective food frequency questionnaires to assess maternal dietary
habits may have resulted in recall bias. To more accurately quantify maternal dietary habits, 24 hour dietary recalls should be administered three times per week (twice on weekdays and once on a weekend to account for variability across the week) (Ma 2009). Furthermore, food frequency questionnaires, used by many of these studies, were either not validated (García-Mantrana 2020) or were validated in a non-pregnant/lactating population (Babakobi 2020; Chu 2016; Lundgren 2018; Savage 2018). Collection and analysis of dietary biomarkers may help in validating the results of self-reported data.

A major limitation of many of the above studies is that maternal and/or infant antibiotic use was not reported nor accounted for (LeMay-Nedjelski 2020; Lundgren 2018; Ponzo 2019; Savage 2018). This is important, considering that perinatal and early-life antibiotic use can dramatically reduce bacterial diversity, and alter the composition of the infant gut microbiome (Bokulich 2016; Modi 2014; Stinson 2018). Notably, only two of these studies reported maternal prenatal/lactational supplement use: Babakobi et al. reported maternal use of omega-3 supplements during lactation (Babakobi 2020) and Savage et al. reported maternal use of vitamin D supplements during pregnancy (Savage 2018). Of particular importance is the fact that many modern prenatal and breastfeeding supplements contain pre- and probiotics, which may affect the results of such studies. Given that supplements may influence the microbiome, a comprehensive assessment of maternal dietary supplement intake is necessary for such future studies.

Another major limitation in all of the above studies is the use of short amplicon sequencing, resulting in limited taxonomic resolution (Johnson 2019b). Future research should utilise long amplicon sequencing or whole genome sequencing to better identify links between maternal diet during gestation and lactation and the infant gut microbiome. Importantly, negative controls were either not used or not reported on in most HM microbiome (Babakobi 2020; Cortes-Macías 2020; LeMay-Nedjelski 2020; Williams 2017) and all of the infant gut
microbiome studies (Babakobi 2020; Chu 2016; Lundgren 2018; Ponzo 2019; Savage 2018). This is of particular concern for studies of HM and other low-biomass samples, which are vulnerable to exogenous contamination (Stinson 2019b). Observational studies such as these are also confounded by the high level of inter-individual variation in the human microbiome. This is made worse by the numerous co-factors which are associated with diet that may also influence the HM or infant microbiomes (such as maternal obesity, socio-economic status, and ethnicity) (Bowyer 2019; Butts 2020; Cerdó 2018; Lundgren 2019; Xu 2020).

Dietary intervention studies should be utilised to provide a more controlled evaluation of the effect of maternal diet, through the use of a well-defined dietary intervention or targeted supplementation. Currently, it is difficult to identify specific dietary factors that should be utilized in future interventional studies, as the existing observational studies have classified food items differently. Pooling the available dietary data from observational studies may assist in identifying targets for future interventional studies. However, it should be noted that it has previously been shown that individual microbiomes display a personalised response to diet (Johnson 2019a). Therefore, it may be necessary to tailor dietary interventions according to individual microbiome composition and genetic factors. Further, it is unknown whether a prolonged effect of a maternal dietary intervention on the infant gut microbiome can be achieved. Short-term dietary interventions in adult humans result in rapid, but transient alterations to the gut microbiome (David 2014). Further well-designed observational studies are currently required to identify dietary factors worth targeting in later interventional studies and to address the issue of duration of intervention.
Evidence from animal studies that maternal diet alters the milk and infant microbiomes

Despite the limitations noted above, the findings of these human observational studies are largely supported by evidence from animal studies (Table 4). Pups of mice fed a high-fat diet during pregnancy and lactation have significant alterations in their gut microbiota, including an increased Firmicutes/Bacteroidetes ratio, an increased abundance of Lachnospiraceae, Clostridiales, Tenericutes and Verrucomicrobia, and decreased bacterial diversity compared to pups of mice fed a low-fat diet (Myles 2013; Wankhade 2017). Similarly, maternal consumption of an obesogenic diet before, during, and after pregnancy resulted in a decreased level of bacterial diversity and abundance of Bacteroides spp. and Blautia spp. in the gut microbiota of both dams and their offspring (Srinivasan 2018). Such changes may predispose offspring to obesity, as a high Firmicutes/Bacteroidetes ratio has been implicated in the development of obesity (Armougom 2009; Koliada 2017; Ley 2006; Million 2012; Santacruz 2010). However, results from other studies are conflicting, which renders the significance of this Firmicutes/Bacteroidetes ratio debatable (Collado 2008; Duncan 2008; Schwiertz 2010). Reduced gut diversity in offspring of dams fed a high-fat diet is also likely be disadvantageous, as low gut bacterial diversity has been associated with poor health (Manichanh 2006; Opstelten 2016). Maternal consumption of a safflower oil diet rich in n-6 PUFAs and a fish oil diet rich in n-3 PUFAs during pregnancy and lactation has also been associated with compositional alterations to the offspring gut microbiome (Gibson 2015). Interestingly, the offspring of dams exposed to a fish oil diet had an increased abundance of potentially pathogenic bacteria such as Bilophila wadsworthia, Enterococcus faecium, and B. fragilis. In addition to the effects of maternal high-fat diets, other dietary components may also influence the infant gut microbiome. In a study by Hallam and colleagues, feeding rat dams a high prebiotic-fibre diet during pregnancy and lactation resulted in increased...
abundance of faecal *Bifidobacterium* spp. in the offspring compared to offspring of dams fed a high-protein diet (Hallam 2014). The association between maternal diet and the composition of the infant gut microbiota is further supported by studies of non-human primates, in which a maternal high-fat diet during pregnancy and lactation has been associated with reduced abundance of non-pathogenic *Campylobacter* spp. in the offspring gut (Ma 2014). Given that this genus is a common feature of the non-human primate gut microbiome (Dassanayake 2005; Whittier 2010), the significance of this finding is unknown. Differences in the offspring gut microbiome were most marked among offspring of mothers exposed to a high-fat diet for the whole of pregnancy and lactation, as well as post-weaning. Importantly, however, when offspring of mothers who consumed a high-fat diet during pregnancy and lactation were fed a control diet (standard chow that contains 13% fat) after weaning, their gut bacterial profiles were still significantly different from the control group, demonstrating that gestation and lactation are critical windows for programming the gut microbiome. Interestingly, Shively et al., have demonstrated that the mammary gland microbiota in non-human primates can be modified by diet, even when the animal is not actively pregnant or lactating (Shively 2018). In this study, consumption of a Mediterranean diet was associated with increased abundance of *Lactobacillus* spp. and decreased abundance of *Ruminococcus* spp. in the mammary gland tissue compared to a high-fat diet. This suggests that the mammary gland microbiota can be influenced by diet even outside of the context of lactation, and may indicate an influx of bacteria from the gut to the mammary gland tissue independent of lactation.

Two studies have investigated the association between maternal dietary fibre intake and the offspring gut microbiota during gestation and lactation in pigs. Leblois et al. reported that piglets of wheat bran supplemented sows had a higher relative abundance of gut *Clostridiaceae* compared to control piglets (Leblois 2017). In a similar study, maternal
supplementation with inulin during gestation and lactation resulted in a decreased cell count of Enterobacteria and *Lactobacillus amylovorus* in the stomach digesta of piglets, and an increased cell count of Enterococcus and *Clostridium leptum* in the caecum digesta of piglets compared to a control group (Paßlack 2015).

As with the human studies conducted in this area to date, the animal studies reviewed here suffer from significant limitations. Most have used short amplicon sequencing methods to characterise the bacterial communities in offspring gut, resulting in limited taxonomic resolution at the genus and species level. Negative controls were not reported in any of these studies. Additionally, all failed to either match or define the micronutrient percentages in the dietary interventions received by control groups and test groups. Most of these animal studies include a prolonged period between maternal dietary intervention and collection of offspring faecal samples. Such an approach tends to overlook the fact that murine gut bacterial composition can be altered rapidly (in as little as three days) in response to diet (Collins 2016). The male to female ratio across the different groups was also either not reported (Gibson 2015; Leblois 2017; Ma 2014; Myles 2013; Paßlack 2015) or significantly different (Hallam 2014). This may have influenced the results given that sex has been reported to influence the composition of the murine gut microbiome, likely driven by hormones (Org 2016). Reduced litter size can also negatively impact offspring, as one study reported slowed postnatal growth and reduced milk intake by offspring who underwent a reduction in their litter size (O'Dowd 2008). A reduction in milk intake may reduce the dose of maternal microbes that the offspring are exposed to, and will therefore likely influence the outcome of the study.

It is also important to recognise the potential influence of the selected animal population in each study. Strain-specific genetic effects have been shown to influence the gut microbiome composition of laboratory rodent models (Campbell 2012; Friswell 2010; Hildebrand 2013).
In general, caution must be applied when interpreting data from animal studies, given the extensively identified limitations in such models (Laukens 2016; Nguyen 2015). Collectively, the animal studies reviewed here provide evidence that maternal diet during pregnancy and lactation can influence the offspring gut microbiota.

**Could dietary intervention during lactation improve infant health?**

The early postnatal period is a critical window for the development of the infant gut microbiome and lifelong health. Therefore, if the maternal diet during lactation alters the infant gut microbiome, it may, in turn, influence the long-term health of the infant. Accordingly, animal and human studies have suggested that the maternal diet during pregnancy and lactation can contribute to the risk of development of metabolic disorders in the offspring later in life (Aaltonen 2011; Ashino 2012; Mennitti 2015; Vogt 2014). To date, there exists only one observational study of the effect of the maternal diet during lactation on the breastfed infant gut microbiome. Nonetheless, the collective evidence suggests that maternal diet may influence the infant gut microbiome via changes to the maternal gut and HM microorganisms. Additionally, it is possible that the influence of maternal diet on the infant gut microbiome may be independent of HM. For example, the maternal microbiome will contribute to the built environment microbiome in the home, and thereby affect infant bacterial exposures (Adams 2015; Leung and Lee 2016; Meadow 2014). Well-controlled studies of exclusively breastfed and exclusively formula-fed infants may, therefore, better elucidate the mechanism by which maternal diet may affect the infant gut microbiome.

In addition to the direct impact on the infant microbiome, modulation of the maternal microbiome with diet may also have indirect effects on infant health via the production of
bacterial metabolites, such as SCFAs. These metabolites are involved in various physiological processes such as host immunity, energy metabolism, and cell-to-cell communication (Russell 2013). Gut bacterial metabolites can also participate in protection against inflammation, autoimmune disorders, cardiovascular diseases, and some cancers (Hijova and Chmelarova 2007; Huda-Faujan 2010; Thorburn 2014). Importantly, SCFAs can have epigenetic effects via their inhibition of histone deacetylases (Fellows 2018), highlighting their potential role in developmental programming of the infant. The profile of SCFAs produced is dictated by the bacterial composition of the gut, and type and quantity of fibre consumed (Baxter 2019; Cook and Sellin 1998; Fredstrom 1994; McIntyre 1991; Raninen 2011). Evidence from murine studies suggests that fibre intake during pregnancy alters the maternal gut microbiome in favour of SCFA-producing bacteria, resulting in increased offspring regulatory T cell numbers and protection from offspring allergic airway disease development later in life. Direct supplementation with acetate in pregnant mice elicited the same effect. Thus, maternal SCFAs supplementation may increase levels of HM SCFAs, potentially conferring health benefits to the developing infant.

The effect of maternal diet on HM SCFA profiles has not been studied; however, HM SCFA profiles differ geographically, suggesting a potential dietary link (Gómez-Gallego 2018; Stinson 2020b). Further research is required to determine the association between the maternal diet, HM SCFAs, and infant health. To date, only one study has investigated the effect of maternal diet on HMO composition (Seferovic 2020). In a randomised cross-over study, seven lactating women received glucose or galactose as the sole source of carbohydrate for 50-57 hours. Another seven lactating women underwent a randomised cross-over study in which they underwent a carbohydrate-rich or high-fat diet for eight days. HMO analysis showed the ability of maternal diet to significantly impact the overall HMO-bound sialic acid and fucose composition, with association with the functional potential of the HM
microbiota. The influence of maternal diet on other microbiome-modulating components of HM, such as AMPs is yet to be explored. In view of all that has been described so far, one may speculate that maternal diet may influence infant health via modifying HM components, including SCFAs, HMOs, and AMPs. Further work needs to be done to establish the extent of such relationships, and their impact on the infant microbiome.

**Conclusion**

Accumulating evidence from human observational and animal studies suggests that maternal diet during gestation and lactation may influence the offspring gut microbiome (Figure 1). Elucidating the influence of maternal diet on the bacterial composition of HM and the downstream effects on the infant gut microbiota will allow assessment of potential impacts of maternal diet on infant health and development. While observational cohort studies are somewhat useful in this regard, they are complicated by high levels of inter-individual variation in the microbiome and habitual diet, and by confounding factors such as host health. The next step in this field will be to use the data from observational cohort studies described within this review (with repeated measures for diet and microbiome) to help inform the design of future interventional studies. This includes the identification of foods that are likely to be the focus of the intervention diets, intervention duration and perhaps most importantly, the study population in which the hypotheses will be tested. Controlled dietary interventions would allow each mother-infant dyad can serve as their own “control”. The results of such studies may allow optimisation of dietary recommendations for pregnant and lactating women to better support infant health.
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Figure 1
### Table 1: Evidence for vertical transmission of maternal gut microbes to the infant gut via human milk.

<table>
<thead>
<tr>
<th>Study</th>
<th>Mother-infant dyads (n)</th>
<th>Sampling time</th>
<th>Method</th>
<th>Taxa shared between maternal gut and HM</th>
<th>Taxa shared between HM and infant gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kordy et al. 2020</td>
<td>20; 5 never-latched, 15 latched</td>
<td>First 3 days postpartum (2 pairs of never-latched) (colostrum), first 3 weeks postpartum (2 pairs of never-latched), 19 days postpartum (1 pair of latched), between day 3-55 postpartum (6 pairs of latched)</td>
<td>16S rRNA gene sequencing (V4 region), and shotgun metagenomics (for 6 pairs only)</td>
<td>Bifidobacterium breve</td>
<td>Bifidobacterium breve</td>
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<td></td>
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<td>Contamination controls: Sequences present in negative extraction and PCR controls were removed</td>
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<tr>
<td>Asnicar et al. 2017</td>
<td>5</td>
<td>3 months postpartum (5 pairs), 10 months postpartum (2 pairs), 16 months postpartum (1 pair)</td>
<td>Shotgun metagenomics</td>
<td>Bifidobacterium breve</td>
<td>Bifidobacterium breve, Veillonella parvula</td>
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<td>Contamination controls: Low yield samples were discarded</td>
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<td>Contamination controls: None</td>
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<tr>
<td>Milani et al. 2015</td>
<td>4</td>
<td>3 and 9 months postpartum</td>
<td>ITS amplicon sequencing, 16S rRNA gene sequencing (V3 region), and shotgun metagenomics (for 2 pairs only)</td>
<td>Bifidobacterium longum subsp. longum</td>
<td>Bifidobacterium adolescentis 1892B, Bifidobacterium dentium 1895B, Bifidobacterium breve 1900B, Bifidobacterium breve 1889B, Bifidobacterium breve 1891B, Bifidobacterium longum subsp. Infantis 1888B, Bifidobacterium adolescentis 1897B, and 10029 Bifidobacterium longum subsp</td>
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<td></td>
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<td>Contamination controls: ITS analysis: OTUs with less than 10 sequences were removed, 16S rRNA analysis: None</td>
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<td>Jost et al. 2014</td>
<td>7</td>
<td>Maternal faeces: 2–8 weeks prepartum Colostrum, human milk, maternal and infant faeces: days 3–6, 9–14, and 25–30 postpartum</td>
<td>Pyrosequencing, 16S rRNA gene sequencing (V5-V6), PFGE, RAPD, REP-PCR</td>
<td>Bifidobacterium breve</td>
<td>Bifidobacterium breve, Staphylococcus epidermidis</td>
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<td></td>
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<td>Contamination controls: None</td>
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</table>
Table 2: Associations between maternal diet and the composition of the human milk microbiome. EPA: eicosapentaenoic acid, DPA: docosapentaenoic acid, DHA: docosahexaenoic acid.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population (n), infant gestational age at delivery, location</th>
<th>Time of maternal diet assessment</th>
<th>Maternal diet assessment</th>
<th>Time of human milk sampling</th>
<th>Methods for human milk microbiome analysis</th>
<th>Maternal dietary variables</th>
<th>Associations with human milk microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babakobi et al. 2020</td>
<td>Healthy lactating women (22), full term infants, Israel</td>
<td>3 months postpartum</td>
<td>Food frequency questionnaire at 3 months postpartum to assess maternal diet during the pregnancy period and the first 3 months of lactation</td>
<td>1 week, 1 month, and 3 months postpartum</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Total polyunsaturated fat</td>
<td>Strapteococcus</td>
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<td>Total monounsaturated fat</td>
<td>Strapteococcus</td>
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<td>Follic acid</td>
<td>Strapteococcus</td>
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<td></td>
<td>Vitamin B12</td>
<td>Strapteococcus</td>
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<tr>
<td>Cortes-Macias et al. 2020</td>
<td>Healthy lactating woman (120), full term infants, Spain</td>
<td>Day 11 (±4) postpartum</td>
<td>Food frequency questionnaire</td>
<td>Day 11 (±4) postpartum</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Carbophydrates</td>
<td>Staphylococcus</td>
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<td>Total protein</td>
<td>Staphylococcus</td>
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<td>Total protein, EPA, DPA, selenium, and zinc</td>
<td>Staphylococcus</td>
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<td>Carbohydrates and polyphosphates</td>
<td>Eubacterium</td>
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<td>Total lipid</td>
<td>Eubacterium</td>
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<td>Total animal protein and saturated fat</td>
<td>Eubacterium</td>
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<td>Total animal protein, EPA, and DPA</td>
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<td>Calcium</td>
<td>Eubacterium</td>
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<td>total fibre, plant protein, and insoluble dietary fibre</td>
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<td>Vitamin A</td>
<td>Enterococcus</td>
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<td>Vitamin D</td>
<td>Enterococcus</td>
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<td>LaMay-Nedelsky et al. 2020</td>
<td>Normoglycemic lactating women (56), lactating women with gestational diabetes mellitus (21), or lactating women with impaired glucose tolerance (16), full term infants, Canada</td>
<td>3 months postpartum</td>
<td>Food frequency questionnaire</td>
<td>3 months postpartum</td>
<td>16S rRNA gene sequencing (V4 region)</td>
<td>Fibre from grains</td>
<td>Bacteroidobacteria and Acanthobacter</td>
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<td>Total fibre</td>
<td>Streptococcus</td>
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<td>Trans fats</td>
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<td>Monounsaturated fat</td>
<td>Acanthobacter and Gemella</td>
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<td>Polyunsaturated fat</td>
<td>Acanthobacter</td>
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<td>Padilha et al. 2019</td>
<td>Healthy lactating women (64), full term infants, Brazil</td>
<td>Day 7 (±3) and 30 (±6) postpartum</td>
<td>24 h dietary recall at day 7 (±3) and day 30 (±6) postpartum to assess maternal diet during the lactation period</td>
<td>Day 11 (±4) postpartum</td>
<td>16S rRNA gene sequencing (V4 region)</td>
<td>Vitamin C during pregnancy</td>
<td>Enterococcus</td>
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<td>Polyunsaturated fat and linoleic fatty acid during lactation</td>
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<td>Sugars during lactation</td>
<td>Pseudomonas</td>
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<td>Vitamin B9 during lactation</td>
<td>Pseudomonas</td>
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<td>B vitamins particularly (B1, B2, and B9) during lactation</td>
<td>Enterococcus</td>
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<tr>
<td>Williams et al. 2017</td>
<td>Healthy lactating women (21), gestational age not reported, USA</td>
<td>Days 3, 5, and 10 (±1) and 1, 2, 3, 4, 5, and 6 months (±1 d) postpartum</td>
<td>24 h dietary recall at days 3, 5, and 10 (±1) and 1, 2, 3, 4, 5, and 6 months (±1 d) postpartum</td>
<td>Days 2 and 5 (colostrum), and 10 (±1 d) and 1, 2, 3, 4, 5, and 6 months (±1 d) postpartum</td>
<td>16S rRNA gene sequencing (V1–V3 region)</td>
<td>Saturated and monounsaturated Fat</td>
<td>Clostridium</td>
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<td>Unsaturated and Polyunsaturated Fat</td>
<td>Propionobacteria</td>
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<td>Total carbohydrates</td>
<td>Firmicutes, Bacteroidetes, and Gemella</td>
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<td>Insoluble fibre</td>
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<td>Total protein intake</td>
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<td>Essential amino acids</td>
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<td>Pantothenic acid</td>
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<td>Riboflavin and calcium</td>
<td>Veillonella</td>
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<td></td>
<td>Thiamin, niacin, folate, vitamin B-6, and chromium</td>
<td>Lactobacillus</td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Associations between maternal diet during pregnancy and lactation and the composition of the infant gut microbiome.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study population (n), infant gestational age at delivery</th>
<th>Time of maternal diet assessment</th>
<th>Maternal diet assessment</th>
<th>Time of infant faecal sampling</th>
<th>Infant faecal microbiome analysis</th>
<th>Maternal dietary variables during pregnancy</th>
<th>Associations with infant gut microbiota</th>
</tr>
</thead>
<tbody>
<tr>
<td>Babakobi et al. 2020</td>
<td>Healthy infants (22), full term, Israel</td>
<td>3 months postpartum</td>
<td>Food frequency questionnaire at 3 months postpartum to assess maternal diet during the pregnancy period and the first 3 months of lactation</td>
<td>1 week, 1 month, and 3 months postpartum</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Contamination controls: None</td>
<td>No statistically significant associations were identified at any of the 3 time points</td>
</tr>
<tr>
<td>García-Mantrana et al. 2020</td>
<td>Healthy infants (86), full term, Spain</td>
<td>1st week postpartum</td>
<td>Food frequency questionnaire in the first week postpartum to assess diet during pregnancy</td>
<td>First 24 h after birth (meconium)</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Contamination controls: None</td>
<td>↑Fibre and vegetable protein ↓Bacteroides</td>
</tr>
<tr>
<td>Ponzo et al. 2019</td>
<td>Infants of mothers with GDM (29), full term, Italy</td>
<td>24–28 weeks gestational and third trimester</td>
<td>3-day food record questionnaires</td>
<td>3–5 days postpartum</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Contamination controls: None</td>
<td>↑↑Animal proteins ↓Fibre and vegetable protein ↓Bacteroides</td>
</tr>
<tr>
<td>Ponzo et al. 2019</td>
<td>Infants of mothers with GDM (29), full term, Italy</td>
<td>24–28 weeks gestational and third trimester</td>
<td>3-day food record questionnaires</td>
<td>3–5 days postpartum</td>
<td>16S rRNA gene sequencing (V3–V4 region)</td>
<td>Contamination controls: None</td>
<td>↑↑Animal proteins ↓Fibre and vegetable protein ↓Bacteroides</td>
</tr>
<tr>
<td>Lundgren et al. 2018</td>
<td>Healthy infants (85), full term and preterm, USA</td>
<td>24–28 weeks gestational</td>
<td>Food frequency questionnaire</td>
<td>6 weeks postpartum</td>
<td>16S rRNA gene sequencing (V4–V5 region)</td>
<td>Contamination controls: None</td>
<td>Normal fat diet ↑Bacteroides</td>
</tr>
<tr>
<td>Savage et al. 2018</td>
<td>Infants of mothers or fathers with a history of asthma or allergy (323), gestational age not reported, USA</td>
<td>10–18 weeks gestational and third trimester</td>
<td>Food frequency questionnaire</td>
<td>3–6 months postpartum</td>
<td>16S rRNA gene sequencing (regular samples)</td>
<td>Contamination controls: CTUs with c&lt;10 reads or present in &lt;10 samples were removed</td>
<td>↑Vegetables and ↓processed meats and deep fried foods ↓Bacteroides and Clostridium</td>
</tr>
<tr>
<td>Otu et al. 2016</td>
<td>Healthy infants (157), full term and preterm, USA</td>
<td>Last month of the third trimester</td>
<td>Food frequency questionnaire</td>
<td>24–48 hours postpartum (meconium) and 0–6 weeks postpartum</td>
<td>16S rRNA gene sequencing (V3–V5 region)</td>
<td>Contamination controls: CTUs present in &lt;5% of samples were removed</td>
<td>Normal fat diet ↓Bacteroides</td>
</tr>
</tbody>
</table>

* Results not reported
### Table 4: Animal evidence for the influence of maternal diet during pregnancy and lactation on the offspring gut microbiome. PUFA: polyunsaturated fatty acid.

<table>
<thead>
<tr>
<th>Study</th>
<th>Period of intervention</th>
<th>Species</th>
<th>Dietary conditions</th>
<th>Offspring gut microbiota</th>
<th>Time of offspring fecal sampling</th>
<th>Methods for offspring fecal microbiome analysis</th>
<th>Contamination controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warren et al. 2019</td>
<td>From the day of breeding, throughout pregnancy and lactation</td>
<td>Sprague-Dawley rats</td>
<td>High-protein diet (20.9%, protein, 53.4% carbohydrates, 5.9% fat) vs low protein diet (10.3%, protein, 66.2% carbohydrates, 6.7% fat)</td>
<td>Ruminococcaceae (high-protein diet)</td>
<td>14 days of age</td>
<td>16S rRNA gene sequencing (V4 region)</td>
<td>None</td>
</tr>
<tr>
<td>Srinivasan et al. 2018</td>
<td>From ~7 weeks before breeding, and throughout pregnancy and lactation</td>
<td>Sprague-Dawley rats</td>
<td>Oligosaccharide diet A (40% fat, 10% protein, 41% carbohydrates), VS obesogenic diet B (59% fat, 14% protein, 27% carbohydrates) VS control diet (13% fat, 22% protein, and 65% carbohydrates)</td>
<td>Clostridium sensu stricto, Bacteroidetes, Biadaducti, Clostridium XIVa, Lactobacillus, Allostipes, Oscillobaeter, and Akkropovotella (obesogenic diet)</td>
<td>19 days of age</td>
<td>16S rRNA gene sequencing (V4 region)</td>
<td>None</td>
</tr>
<tr>
<td>Labrois et al. 2017</td>
<td>From day 3 after artificial insemination, throughout pregnancy until 28 days postpartum</td>
<td>L. lactis subsp. lactis</td>
<td>During gestation: High wheat bran diet (15.3% wheat, 25% wheat bran, 7.22% fat, 14.59% crude protein) VS control diet (19.9% wheat, 2% wheat bran, 5.53% fat, 14.55% crude protein) During lactation: High wheat bran diet (17.94% wheat, 14% wheat bran, 6.97% fat, 17.4% crude protein) VS control diet (23.95% wheat, 6.12% fat, 18.08% crude protein)</td>
<td>Unclassified Clostridiales (high wheat bran diet)</td>
<td>26 and 27 days of age</td>
<td>16S rRNA gene sequencing (V3/V4 region)</td>
<td>None</td>
</tr>
<tr>
<td>Wardhede et al. 2017</td>
<td>From 12 weeks before breeding and post-weaning (14 weeks)</td>
<td>C57BL/6J mice</td>
<td>High-fat diet (45% fat) VS control diet (17% fat)</td>
<td>Alpha-diversity, Eubacteriaceae, Peptococcaceae, Tenericutes, and Verrucomicrobia (high-fat diet)</td>
<td>12 weeks after high-fat diet</td>
<td>16S rRNA gene sequencing (V3 region)</td>
<td>Control: None</td>
</tr>
<tr>
<td>Olsson et al. 2015</td>
<td>Pregnancy and lactation</td>
<td>Sprague-Dawley rats</td>
<td>High-fat diet (high in n-6 PUFA)s VS fish oil diet (high in n-3 PUFA) VS control diet (8:1 ratio of n-6:n-3 PUFA)</td>
<td>Bacterial richness, Firmicutes, Bacteroidetes ratio, Enterobacteriaceae, Bifidobacterium, Lachnospiraceae, Clostridium cocoides, Bacillus, and segmented filamentous bacteria (both safflower oil and fish oil diets compared to control diet) VS fish oil diet</td>
<td>15 days of age</td>
<td>PCR</td>
<td>Control: None</td>
</tr>
<tr>
<td>Patlaack et al. 2015</td>
<td>Pregnancy and lactation</td>
<td>M. musculus</td>
<td>During gestation: Inulin diet (2.0% inulin) VS control diet (1.5% inulin) During lactation: Inulin diet (2.2% inulin) VS control diet (0.8% inulin)</td>
<td>Eubacteriaceae and Lactobacillus amylovorus, Enterococcus and Clostridium leptum (inulin diet)</td>
<td>10 days of age</td>
<td>PCR</td>
<td>Control: None</td>
</tr>
<tr>
<td>Hallam et al. 2014</td>
<td>From 1 week before breeding, and throughout pregnancy, lactation, and post-weaning (21 days)</td>
<td>Wistar rats</td>
<td>High prebiotic-fibre diet (corn starch 200g/kg, casein 173 g/kg, inulin/108 g/kg, and oligofructose 108 g/kg) VS high-protein diet (corn starch 197 g/kg and casein 400g/kg) VS control diet (no fibre, 50% less protein)</td>
<td>Unclassified Clostridiales and Lactobacillus (high-protein diet) VS fish oil diet</td>
<td>5 and 24 weeks of age</td>
<td>PCR</td>
<td>Control: None</td>
</tr>
<tr>
<td>Ma et al. 2014</td>
<td>Pregnancy, lactation, and post-weaning (6-7 months)</td>
<td>M. fasciculata primates</td>
<td>High-fat diet (35% fat from land, butter, animal fat, and safflower oil) VS control diet (13% fat from soya bean oil)</td>
<td>Fecalobacterium, Esteredolobacter, Campylobacter, Helicobacter, Firmicutes, Ruminococcus, and Dialister (high-fat diet) VS faecalobacterium (control diet)</td>
<td>1 year of age</td>
<td>PCR and 16S rRNA gene sequencing (V3-V5 region)</td>
<td>Control: None</td>
</tr>
<tr>
<td>Myles et al. 2014</td>
<td>1 day before breeding until 3 weeks postpartum</td>
<td>BALB/c mice</td>
<td>High omega-3 (n-3 PUFA) diet (40% carbohydrates and 40% fat) VS control low-fat diet (70% carbohydrates and 10% fat)</td>
<td>Bifidobacterium, Lactobacillus, Clostridiales, Robisoniella, Lactococcus, Enterococcus, Anaerotunrillus, and Roseburia (high omega-3 diet)</td>
<td>1 week after weaning</td>
<td>16S rRNA gene sequencing (V1-V3 region)</td>
<td>Control: None</td>
</tr>
<tr>
<td>Myles et al. 2013</td>
<td>Pregnancy and lactation</td>
<td>BALB/c and C57BL/6 mice</td>
<td>Western diet (20% protein, 40% carbohydrates, and 40% fat) VS control low-fat diet (20% protein, 70% carbohydrates, and 10% fat)</td>
<td>Firmicutes, Bacteroidetes ratio, Lachnospiraceae, Clostridiales, and Alpha diversity (Western diet)</td>
<td>1 week after weaning</td>
<td>16S rRNA gene sequencing (V1-V3 region)</td>
<td>Control: None</td>
</tr>
</tbody>
</table>