A narrative review of the functional components of human breast milk and their potential to modulate the gut microbiome, the consideration of maternal and child characteristics, and confounders of breastfeeding, and their impact on risk of obesity later in life.

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Graphical Abstract

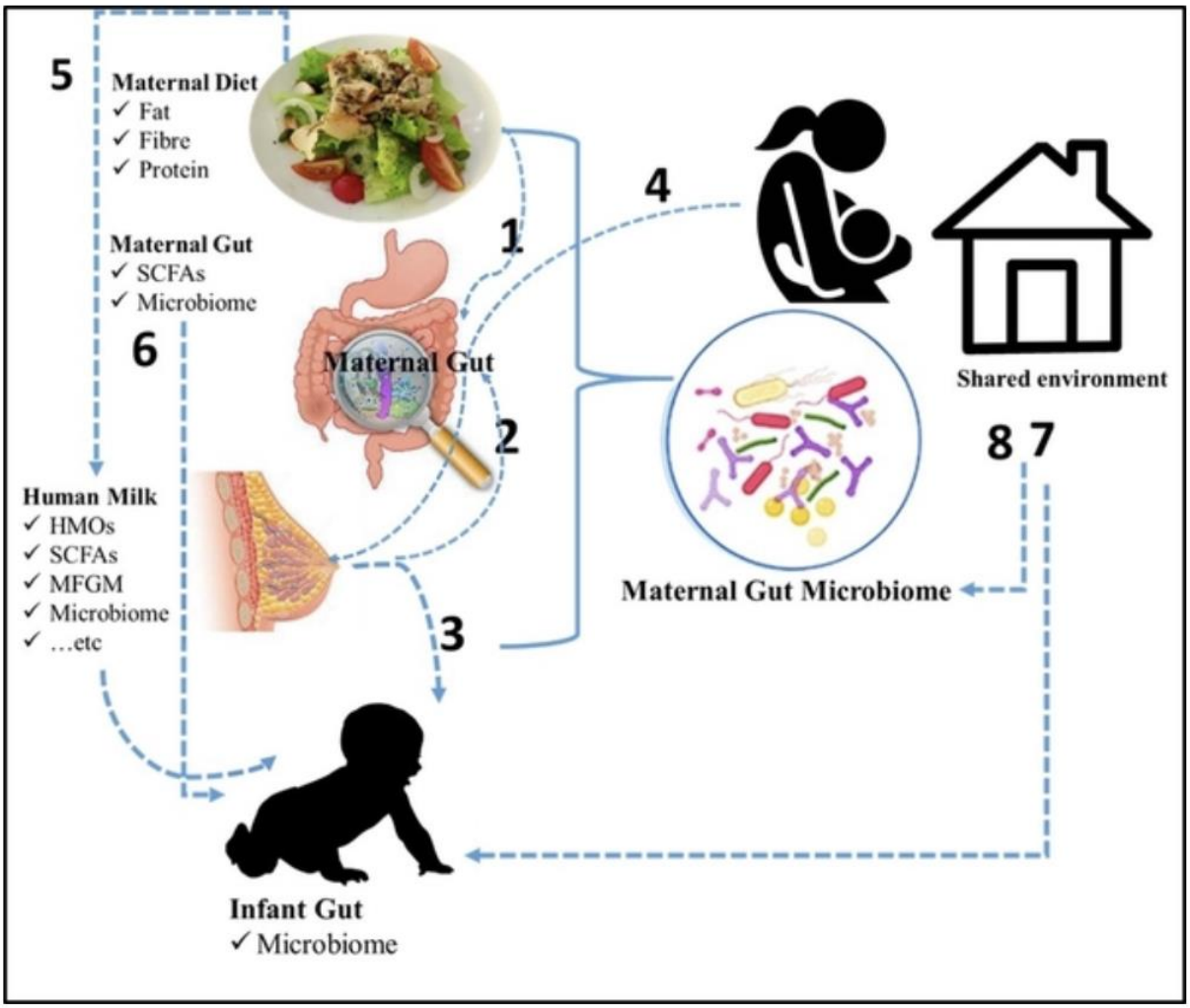

Figure 1. A schematic representation of the proposed links between maternal diet, maternal gut, human milk, and infant gut microbiomes. (1). Maternal diet can affect the composition of the gut microbiome. (2) Some bacteria are suggested to be transferred from maternal gut to milk via an entero-mammary pathway. Some bacterial metabolites are transferred to and acting as prebiotics in the milk (3) Human milk can shape the infant gut microbiome via direct transfer of bacteria and exposure to microbiome-modulating factors such as SCFAs, and HMOs. It is worth noting that both bacteria and microbiome-modulating factors are produced by the mother and via maternal gut microbiota. (4) Maternal diet has been associated with the human milk microbiome. (5) Some studies support the association between maternal diet during pregnancy and the infant gut microbiome. (6) Maternal gut microbes may be transferred to the infant gut independent of breastfeeding. (7, 8) A Sharing of bacteria between mother and infant may occur as a result of a shared environment. Even though there may be an existence of shared taxa in infant and mother, this may not necessarily indicate a vertical transfer. This figure has been adapted from the work of Sindi et al 2021.52

ABSTRACT

Nutritional exposure and therefore the metabolic environment during early human development can impact on health later in life. This can go beyond the nutrients consumed, as there is evidence that the development and modulation of the gut microbiome during early lifecan have an impact on human growth, development and health, which is associated with the risk of obesity later in life. The primary aim of this review was to evaluate existing evidence; to identify the components of human breast milk which may modulate the gut microbiome and the impact on the risk of becoming obese later in life. This review also considers maternal and child characteristics, and confounders of breastfeeding and how they impact on the infant gut microbiome. Current evidence supports a positive association between faecal branched short-chain fatty acids and human milk oligosaccharide-diversity and a gut microbiome associated with better metabolic health. A negative correlation was found between microbiome diversity and human milk oligosaccharide evenness which was associated with a greater fat mass and percentage fat. The components of human breast milk including: oligosaccharides, probiotics, milk fat globule membrane and adiponectin were hypothesised to positively influence infant growth and body weight by modulating the microbial diversity and composition of the gut. Maternal diet, timing and duration of breast feeding in addition to the mode of delivery were all shown to impact on the human milk microbiota. However, further experimental studies with long duration of follow-up are required to shed light on the governing mechanisms linking breast milk components with a diverse infant microbiome and healthier body weight later in life.

Keywords: Gut microbiome, Human or breast milk, Human milk oligosaccharides, Milk fat globule membrane, Obesity

Introduction

Human development during first months of life represents an important opportunity to prevent obesity and its adverse consequences later in life.[1] Among the modifiable risk factors for childhood obesity in the first 1,000 days of life, breastfeeding has a strong and consistent body of evidence to support its roles as a protective factor with respect to health later in life.[2]

A growing body of evidence has begun to suggest the link between the gut microbiome and risk of obesity,[3] with most studies reporting an increase in the ratio of *Firmiticus* to *Bacteroidetes* in adults living with obesity compared with the lean counterparts. [4,5] Breast milk (BM) or human milk (HM) has been seen as one of the main drivers of microbial colonisation of the gut early in life.[6] BM, which is the biological norm of infant's nutrition, has been reported to contain several bioactive compounds which are associated with the formation of a healthy gut microbiome[6]. In addition, BM or HM which is rich in *Bifidobacteri*a, have been reported to be present to a lesser extent in the gut of children living with obesity. [7] An experimental study by Kalliomäki et al. (2008) observed that children who were overweight at 7-years-old had lower levels of *Bifidobacteria* at the age of 6 and 12 months.[8]

Although studies on early human gut microbial populations are scarce, the association between the infant gut and the risk of childhood obesity is well documented. An early perturbation of the infant gut microbiome has been postulated as a risk factor for metabolic disorders including obesity.[9] Tanaka and Nakayama (2017) found evidence to suggest that a stable gut microbiome can be establishedduring two crucial transitions in infancy: one after birth with the initiation of breastfeeding (or formula) and the second during the introduction of complementary foods alongside breastfeeding (or formula) ('weaning'). The perturbation of this 'critical window' (two transitions) has long-term effects on individuals' health.[10]

When a mother is not able to breastfeed her infant, formula milk (FM) is used as a substitute. Even though significant development work has been undertaken in an attempt to ensure that FM mimics BM, FM still lacks several bioactive ingredients such

as human milk oligosaccharides (HMOs), milk fat globular membrane (MFGM), and proteins such as lactoferrin. It is thus hypothesised that the differences in composition between BM and FM may have an impact on the infant microbiome.

From an ecological point of view, the colonization of the infant's gut does represent the *de novo* assembly of a microbial community [11]and is influenced by dietary factors such as breast or formula feeding as well as medical factors.[12]

The link between breastfeeding and the nature and type of gut microbiome and how it impacts health is an area of emerging interest. As aforementioned, the gut microbiome of breastfed infants is dominated by *Bifidobacteria* species. In contrast to this, formula-fed infants have a more diverse gut microbiome.[13] Strikingly, formula-fed infants have an increased risk of being overweight than their breastfed counterparts.[14] Therefore, there has been a consensus that FM increases the risk of later obesity while BM confers protective effects. However, even though many studies have examined the influence of early nutrition on later obesity risk, there is a paucity of knowledge on how the various components of BM have the potential to modulate the gut microbiome and infant weight parameters and how this might influence the risk of developing obesity in adulthood. Therefore, this review aims to investigate how BM composition modulates the gut microbiome and how this influences infant weight gain and its impact on obesity later in life. Additionally, this review considers the impact of maternal and child characteristics, and confounders of breastfeeding and their impact on the composition of BM microbiome. To our knowledge, no previous reviews have considered the effect of different BM components on the infant's gut microflora and its potential to influence infant growth and or body weight, especially with respect to the potential mechanisms which attempt to explain the association of its effect on infant gut microbiome and influences on body weight.

Methods

To initially consider how BM modulates the gut microbiome and its impact on infants' weight, data from animal models which involved the feeding of the milk

containing possible components of breastmilk as well as data from human studies was evaluated. The data was obtained by undertaking a search of seven electronic databases (AMED, CINAHL, APA PsycArticles, MEDLINE, APA PsycINFO, Education Source and PubMed) from inception to May 2021.The following key words were used: breast milk, protein, human milk oligosaccharide, milk fat globule membrane, *Enterococci, Bifidobacterium*, infant microbiome and obesity or weight gain or adiposity. The findings from these databases were then summarised in to **Table 1**[15,16] which shows the effect of components in breast milk and how they impact on various growth parameters in piglets and rat pups, and **Table 2**[17-25] which presents data on breastfeeding from human studies.

Results

Human breast milk (BM) is composed of human milk oligosaccharides, milk fat globule membrane, short chain fatty acids and probiotics among others organic compounds, which have the potential of modulating the infant gut microbiome. BM is also composed of 90 + % water and 0.2 % ash (minerals) which do not fall in the scope of this review, and have not be considered.

The proposed bioactive components identified in BM (human milk oligosaccharides, probiotics, milk fat globular membrane, adiponectin, leptin, glucagon-like peptide-1 and Peptide YY), their impact on the gut microbiome and the role that they play in the infants' growth as demonstrated by the data available from both the animal (**Table 1**)[15,16] and human studies (**Table 2**)[17-25] will be discussed in subsequent sections.

Discussion

Human milk oligosaccharides (HMOs)

The role of individual HMO levels have been associated with infant growth trajectories,[17] weight-for-length Z scores (WLZ), crown-to-heel length, nude body weight, and the relative percentage of fat and absolute total body composition after controlling for maternal BMI. This suggests a plausible role for HMOs in influencing infant growth. The amount, precise concentration and the type of HMOs have been shown to vary depending on (i) the duration of lactation, (ii) the genetic makeup of an individual woman, and (iii) the potential environmental exposures.[26]

One of the most prominent relations between HMOs and infant body composition was reported for lacto-N-fucopentaose I (LNFPI)(**Table 2**).[17] At 1-month, LNFPI was inversely associated with infant weight, whereas at 6-month, LNFPI was inversely associated with infant weight, lean mass, and fat mass.[17] LNFPI has been associated with a lower weight, lower lean mass, and a lower fat mass. Notably, at 1- and 6-month LNFPI explained 18 % and 6 % more of the variance in infant weight at each time point than did other significant covariates such as maternal pre-pregnancy body mass index (BMI) and pregnancy weight gain. In contrast to Alderete et al. (2015),[17] Davis and colleagues (2017)[18] found that fucosylated HMOs such as did not predict infant weight and weight-for-age Z-score (WAZ), but the HMO 3' sialyllactose (3'SL) was the strongest predictor positively correlated with WAZ.[18]

Larsson and co-workers (2019) reported a positive correlation between the total HMO concentrations and the total HMO-bound fucose at 5 months and the fat mass index and the weight velocity from 0 to 5 months (**Table 2**).[19] Contrastingly, no associations were found between 2'FL BM concentrations and infants' weight and height velocities in infants consuming either milk with high or with low 2'FL concentrations.[19] Previous findings have established a causal microbiota-dependent relationship between sialylated oligosaccharides and offspring growth via an enhanced ability to utilise nutrients for anabolism. Several hypotheses have been put forward to explain why HMOs play a significant role in the modulation of the gut microbiota and how it impacts infant growth. Firstly, it has been postulated that HMOs are minimally digested in the gastrointestinal (GI) tract, and as such, they end up reaching the colon intact, where they modulate the microbiota.[27,28] Thus, HMOs reach distal parts of the infant's GI tract, where they become metabolised by the colonic microbiota. Secondly, Plows et al. (2020) suggests that the composition of HMO can influence infant feeding behaviour, which could directly influence infant growth via changes in caloric intake.[24] Thirdly, Saben et al. (2021) observed HMOs and HMO metabolites in the urine of breastfed infants, suggesting that some HMOs may be absorbed and have systemic effects on infant physiology as they may bepartly absorbed intact as metabolites from the gut microbiome into circulation.[29] Fourthly, some

authors have suggested that many complex carbohydrates cannot be digested by the host. However, the gut microbes preset within the host can metabolize these carbohydrates to short chain fatty acids (SCFA), such as acetate, butyrate, and propionate.[30] Propionate and acetate are necessary for lipogenesis and gluconeogenesis in the liver whereas butyrate is mainly used as the primary energy source for colonic epithelial cells.[31]Differences in SCFA levels have been observed in lean and obese mice. For instance, in a genetic model of obesity, obese mice were reported to have increased butyrate and acetate concentrations in their ceca and less energy, compared to their lean counterparts.[30]

It is widely accepted that factors, such as maternal weight and body composition, have been linked to growth in infancy. It is possible that maternal size might influence breastmilk composition. Lacto-N-hexaose (LNH) and difucosyllacto-N-tetrose (DFLNT)concentrations were higher in overweight and mothers living with obesity than in mothers who were a normal-weight (**Table 2**).[24] These findings show that HMOs are essential in the gut microbiome modulation, which have evidence for a concomitant impact on infant growth and weight gain.

Probiotics and the gut microbiome

Experiments that involved microbiota transplantation have shown the accumulation of body fat to be dependent on the type of the gut microbiota, which supports the role that is played by the gut microbiota in the development of obesity.[32] Even though the link between the gut microbiome and obesity is not yet well established, some research suggested that through the understanding of the role of the gut microbiome in weight and health management may lead to future improvements in the care and management of obesity.[21] BM has been shown to contain several probiotics that can contribute to the diversity of the gut microbe. An inverse correlation between *Enterococcus* abundance and age/gender-adjusted body weight, waist circumference and BMI levels of plasma leptin and body fat has also been observed (**Table 2**).[21] Thus, probiotic compounds in BM have been hypothesised to have the potential to inhibit fat accumulation, reduce inflammation and insulin resistance, and regulate neuropeptidesand gastrointestinal peptides.

A crucial determinant of gut microbiota development is the mode of infant feeding.[7] The relative abundance of several gut microbiome organisms have been reported to be influenced by the duration of exclusive breastfeeding rather than age at the introduction of complementary feeding. Exclusive breastfeeding was positively correlated with higher concentrations of *Bifidobacteriacea*e and *Veillonellaceae*. These species are known to utilise plant-derived complex carbohydrates and resistant starch which would be introduced when complementary feeding is initiated. Additionally, exclusive breastfeeding was positively correlated with *Bifidobacteriaceae*, known to utilise the lactose and human milk oligosaccharides found in BM.[20]

Previous animal studies suggested that *Enterococci* could have anti-obesity effects.[33,34] *Enterococci* may reduce obesity through the activation of the brown adipose tissue (BAT). BAT is a thermogenic tissue able to convert macronutrients as heat, thus potentially reducing the deposition of fat. Hence, its activation has the potential of increasing the energy expenditure and moderating weight gain.Furthermore, dysbiosis has been associated with additional energy being harvested per day.[35] A 20 % increase in *Firmicutes* and a corresponding 20 % decrease in *Bacteroidetes* resulted in an additional 150 Kcal of energy harvested per day in adults.[35] Despite most studies reporting data from adults, a higher amount of Firmicutes has also been found in formula-fed infants than their breastfed counterparts, [36] which suggests that this may occur throughout life. *Firmicutes* are the primary contributors of butyrate while acetate and propionate are mainly produced by *Bacteroidetes*.[37] A change of about 20 % relative abundance of *Firmicutes* and a corresponding decrease of *Bacteroidetes* has the potential of resulting in approximately 150 kcal energy harvesting difference in the stool of lean individuals.[32]

The data with respect to the modulation of the gut microbiome suggests that maternal obesity per sé does not influence infant gut microbiome development during the initiation of complementary feeding. Furthermore, a high gut microbiome similarity between two cohort studies was observed.[20] In these two cohort studies, the body composition of breast fed babies were independently sampled during different periods, allowing a high-powered characterisation of the infant gut microbiota development and identification of the

main factors explaining variation in gut microbiota. The breastfeeding duration and the composition of the complementary diet, rather than the timing of the introduction of complementary foods, were the main determinants of the gut microbiota composition during late infancy.

Milk fat globule membrane (MFGM)

Data suggests that MFGM might be protective against obesity by contributing to the modulation of the gut microbiome.[38] Therefore, MFGM has been hypothesised to have the potential to reduce the risk of developing obesity through promoting beneficial bacteria in the infant's gut. The mechanisms of action explaining any anti-obesity effect of MFGM are unclear, but they may imply the ability to moderate alleviations of metabolic endotoxemia.[39] Metabolic endotoxemia is a condition characterised by elevated plasma lipopolysaccharides (LPS).[40] LPS are endotoxins constituting the outer membrane of gram-negative bacteria.[41] To date, the available data is supportive that endotoxemia effectively contributes to inflammation, insulin resistance, and the onset of obesity,[41] therefore reducing endotoxemia could plausibly reduce risk of developing obesity.

Gong et al. (2020) reported (**Table 1**)[16] that formula milk (FM) reduced BW in an animal model (rat pups) compared with breastfed rat pups. However, the addition of MFGM to FM reversed this weight trend as showed by elevated BW, narrowing the gap with the breastfed group and suggesting the beneficial properties of MFGM on body composition. The findings of Gong et al. (2020) are in agreement with those of Berding et al. (2016), who reported BW improvements in a different animal model (neonatal piglets) (**Table 1**)[15,16] consuming FM containing the bioactive compound (TEST diet containing MFGM). Between day 17 and the end of the study period, piglets consuming the TEST diet (with MFGM) showed a significant increase in BW ($p < 0.05$) compared with the CONTROL diet. At a glance, the results of these two studies may seem to be contradictory as there is an increase in the body weight in breast fed infants or in FM fed infants when the FM was supplemented with MFGM. However, healthy and steady body weight gain should be the norm during the early stages of life, with infants fed with BM being reported to grow more

rapidly during the first 1 - 2 months and then more slowly both in terms of weight gain and linear growth in the first years.[42] The hypothesis put forward to explain the role of MGM on infant growth proposes beneficial effects of MFGM on bacterial colonisation. The gut flora induced by MFGM might increase nutrient absorption and eventually improve BW by ensuring the healthy development of the intestinal barrier.[15]

MFGM supplementation at least in animal models is able to modulate intestinal flora.[15,16] Gong et al. (2020) demonstrated that MFGM supplementation increased *Firmiticus, Bifidobacteria,* and *Lactobacillus.* Importantly, all three are gram-positive bacteria, were increased in their abundance, and this could result in a reduction of LPS from gram-negative bacteria. This in turn could explain the alleviation of metabolic endotoxemia and consequently increased risk of developing of obesity. On the contrary, a lower abundance of gram-negative bacteria such as *Escherichia/Shigella* was observed in the TEST piglets compared with the CONTROL.[15] However, noshifts were detected in the abundance of either *Bifidobacteria* or *Lactobacillus* when the TEST milk was supplemented with MFGM and a combination of other bioactive ingredients, including lactoferrin and HMOs. Therefore, the mixture of breastmilk components might attenuate the efficacy of MFGM and its effects on *Bifidobacteria* and *Lactobacillus.* A correlation between the differences in faecal microbiota of infants (6 and 12 months) and the risk of being overweight or obese at 7 years of age 16 has been reported.[30] Children of normal weight were shown to have a higher *Bifidobacterial* and lower *Staphylococcus aureus* concentrations at ages 6 and 12 months compared to children who became overweight/obese,[8] suggesting that differnces in the microbiota precede overweight/obesity. Overall, the data to date suggests that MFGM is necessary for healthy infant weight given that MFGM may encourage the development and maintenance of a beneficial bacteria gut microflora. This could have the potential to improve metabolic endotoxemia by reducing the plasma levels of LPS and encourage the development of the intestinal barrier along with improved nutrients absorption. Nevertheless,more research is needed in order to be able to validate this hypothesis, given the experimental stage of research on MFGM. Alike,

the hypothesis for the role played by MFGM on the infant gut microbiome, and BW remains open as more research is needed on MFGM andits impact on obesity later in life.

Other breast milk components (Adiponectin, Leptin, Glucagon-like peptide-1 (GLP-1), Peptide YY (PYY))

Other components in breast milk (BM) may also have the potential to influencing an infant's growth rate without modulating the gut microbiome. Examples include hormones such as leptin, adiponectin, GLP-1, and PYY, which are found in BM and are known to influence food consumption and weight gain. As such, these constituents of BM could influence an infant's growth rate. The hormone leptin, which is produced in adipose tissue, has been detected in BM, and their concentration in BM was significantly correlated with the mothers' BMI.[43] Leptin decreases food intake and increases energy expenditure through the hypothalamic regulation of energy homeostasis. [44] Even though Brunner et al. (2014) found that leptin concentrations at 4 months were inversely associated with infant BMI and lean body mass, after a follow-up, the correlation was lost.[45] These observations are in line with Miralles and colleagues [46] and Uysal and co-workers,[43] who did not find a significant correlation between leptin concentrations in BM and infant weight gain. Leptin concentrations were however reported to result in a significant reduction ($P < 0.01$) in the early pioneering bacteria *Gammaproteobacteria* and exhibited a trend for higher total SCFA content.[47]

In contrast, the true nature of the relationship between milk adiponectin and infant growth seems to be unclear as there is conflicting evidence relating adiponectin levels in BM to adiposity. For instance adiponectin levels in early infancy were related to higher fat mass and more significant weight gain in children up to 2 years.[45] Contrastingly, Woo and co-workers [48] showed adiponectin to be associated with lower infant adiposity by 6 months of age. The effect of adipokines on infant metabolism and growth depends on their questionable bioavailability due to their molecular size. Weight loss was most strongly associated with high molecular weight adiponectin (the form appears to be the most biologically active) and

circulating high-density lipoprotein cholesterol than total adiponectin.[48] An inverse correlation between circulating adiponectin and obesity in children has been reported.[49]

Schueler et al. (2013) measured a range of appetite hormones GLP-1, PYY, and leptin in BM and correlated these with measures of infant growth. A negative correlation was observed between GLP-1 concentration and infant weight gain over the first 6 months ($r = -0.67$, $p = 0.034$; $n = 10$) and the infant weight-for-length percentile at 6 months ($r = -0.64$, $p = 0.046$; $n = 10$). No correlations were observed regarding leptin or PYY.[50] It can be concluded that there is no strong correlation between leptin, GLP-1, and PYY and infant weight gain. However, some evidence suggests that adiponectin might play a role in the modulation of infant weight gain.

Consideration of maternal and child characteristics and confounders of breastfeeding

Even though breastfeeding has been reported to impact the infant gut, which in turn has been shown to affect the growth and development, there remains to be a number of maternal and child characteristics and other confounding factors that affect the composition of BM which have a concomitant effect on infant growth and development and could be linked to obesity later on in life. These factors will be expounded on in the subsequent sections.

Maternal and infant characteristics and the impact on the gut microbiome and the growth and development in infants

Research by Cabrera and co-workers[11] showed the maternal weight to impact on the diversity of the gut microbiome. Milk from obese mothers was reported to contain a less diverse and different bacterial community compared with milk from normal-weight mothers. Milk from mothers who underwent non-elective caesarean delivery contained a different bacterial community than did milk samples from individuals who gave birth by vaginal delivery or via elective caesarean.[11] Caesarean section has been show to increase the risk of postpartum infection and as such, prophylactic antibiotics have commonly been administered which are reported to reduce the incidence [51] and can pass in some degree into the breast milk. The use of antibiotics during early life can induce lasting effects on the body composition by altering the

intestinal microbiota [30] and this can also impact on the body composition later on in life. A previous animal study showed that mice whose mothers were treated with penicillin before the birth of the pups and throughout the weaning process had a markedly altered body composition in adulthood, with increased total and fat mass.[30]

An association between the infant gut microbiota and maternal gut microbiota has also been found as illustrated in **Figure 1**[52]. Somestudies support the theory of vertical transmission of maternal gut bacteria to the infant gut via human milk.[52] Metagenomics and strain level analyses have reported a shared bacterial strains in maternal faecal/milk samples and infant faecal samples.[53] However, there is a general consensus that among the many bacterial species found in infant stool and human milk, only a limited number of bacteria are equally shared in mother-infant dyads.[54] In particular, *Bifidobacterium* species were consistently identified as one of the shared features of the maternal gut, human milk and infant gut microbiomes.[55] Specific strains of *Bifidobacteria* have been shown to play a significant role in energy metabolism and as such were hypothesised to be helpful in management of obesity.[56] Even though the sample sizes of the studies that have been carried out evaluating the link between maternal gut, human milk, and infant gut microbes are relatively small [53]and in some cases no controls were included, the consistency of the reported findings supports the hypothesis of strain sharing between mothers and infants via human milk.[55]

Effect of storage and collection of breast milk samples on bioactive compounds

There have been a number of initiatives that have been introduced to enable infants who are not able to be breastfed to be able to access breast milk. Currently, pasteurized donor (bank) human milk (DHM) is preferred over premature infant formula for premature infants whose mothers are unable to provide an adequate supply of milk.[57] DHM is mainly provided by women who have delivered at term and donate their milk in later stages of lactation which can range from weeks to several months after delivery.[58] Studies have shown milk donated in later stages of lactation is characteristically low in fat, protein, and other bioactive molecules in comparison to milk that produced during the first weeks after delivery.[59] DHM composition is also affected by the processing of expressed milk which follows stringent protocols applied in HM banks that involve pasteurization, which is necessary for minimizing the potential to

transmit infectious agents in addition to freezing and long-term storage.[60,61] Even though some authors have suggested that the macronutrient composition of DHM to remain relatively intact, there has been a general consensus that shows that the presence of several bioactive components is compromised or destroyed.[58] DHM-fed preterm infants showed differences in the microbial patterns as compared to infants who were fed their own mothers milk. DHM in comparison to the use of formula milk was reported to result in lower rates of weight gain, linear growth, and head growth. If DHM results in lower weight gain, linear growth, and lower head growth, one would wonder how this does correlate to obesity later in life. Some authors have reported HM to have lower energand protein content compared to formula, which resulted in slower weight gain during infancy for infants who were fed with human milk versus formula milk. Faster infant growth has been associated with later obesity in many studies.[62,63] Additionally, accelerated growth in infancy has been hypothesised to result in hormonal changes that program a higher set point for appetite, leading to higher food intake throughout life.[64]

However, changes in the bacterial composition between DHM and infants who had been fed their own mother milk were smaller than in comparison to formula fed infants.[65] Significantly higher abundances of *Staphylococcaceae* (*Staphyococcus* genus) and *Pasteurellaceae*members were found in preterm than full milk. The total human milk oligosaccharides (HMOs) in preterm milk were found to be insignificantly higher concentrations than full term milk.[58] Non-digestible HMOs, have been postulated to (i) have the potential of modulating the composition of microbial communities in the gut, ii) select for beneficial bacteria, and iii) are emerging as early mediators in the relationship between the development of the gut-microbiome in early life and clinical outcomes.[58]

Timing and duration of breastfeeding

Some authors reported the relative abundance of several gut microbiome to be influenced by the duration of exclusive breastfeeding rather than age at the introduction of complementary feeding. Exclusive breastfeeding was positively correlated with *Bifidobacteriacea*e and *Veillonellaceae*. These species are known to utilise plant-derived complex carbohydrates and resistant starch introduced with solid foods. Additionally, exclusive breastfeeding was

positively correlated with *Bifidobacteriaceae*, known to utilise the lactose and human milk oligosaccharides found in BM.[20]

The breastmilk microbiota is reported to evolve over the period of breastfeeding. For instance, there is a higher microbiota diversity in colostrum with *Staphylococcus, Streptococcus, Weisella, Leuconostoc* and lactic acid bacteria (*Lactobacillus* and *Streptococcus*) being the most abundant than mature milk.[11] As soon as 3 - 4 days after birth, the gut microbiota of infants begins to resemble the colostrum microbiota,[66] followed by a gut microbiota rich in *Bifidobacteria* and *Lactobacilli*.[12] After 1 month, the abundance of *Staphylococcus*was significantly reduced, while the lactic acid bacteria (*Lactobacillus* and *Streptococcus*) still being highly abundant. The cessation of breast-feeding was shown to have a great impact on the gut microbiota in 12-month-old infants which coincided with the gut microbial ecology shifting toward an adult-like composition that is enriched in *Anaerostipes, Bilophila*, *Clostridium, Bacteroides*, and *Roseburia*. Contrastingly, the gut microbiome of infants breast-fed at 12 months was still dominated by *Bifidobacterium, Collinsella*, *Lactobacillus*, *Megasphaera*, and *Veillonella*, bacteria that have previously been found in breast milk. Consistently, the "microbiota age" of these 12 month olds was shown to appear younger compared to infants who were no longer breast-fed.[12] These findings highlight the role of breast-feeding in the shaping and succession of gut microbial communities during the first year of life.

Impact of maternal nutrition on the diversity and composition of breast milk microbiota

The mechanisms by which breast milk microbiota is modulated remains to be a matter of debate. There are several hypotheses that have been put forward with the aim of understanding the factors that influence the breast milk microbiota composition. One hypothesis suggests that the human milk microbiota is shaped by the mother's diet with the impact of specific nutrients from the maternal intake on nutrient composition of breast milk being reported.[67,68]An alternative hypothesis has proposed an entero-mammary pathway being a route for bacteria to reach the human milk, and this facilitates the transfer of bacteria from maternal gastrointestinal tract to the mammary gland.[69,70] Other authors have argued that

maternal diet might influence the human milk microbiota diversity as a result ofchanges in the maternal gut microbiota diversity.[71] Research has been carried out to establish the association between maternal diet and the microbiota found in breast milk.[71,72] A significant impact of the intake of specific macronutrient and micronutrient on microbial communities in breast mik from healthy lactating women has been reported.[73] For instance, a higher presence of the *Staphylococcus* and *Bifidobacterium* genera was observed in breast milk where maternal diet was charaterised by a higher intake of proteins from plant origin, dietary fiber, carbohydrates, and polyphenols. Moreover, maternal diet that was characterised by a higher fiber and plant protein intakes, was shown to result in a higher breast milk richness and microbial diversity than breast milk samples from maternal diet characterised by a higher intake of proteins from animal origin, total lipids, saturated fatty acids, and total poly and mono unsaturated fatty acids.[72] The intake of high quantities of proteins from animal origin and fats such as n–3 polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) was negatively correlated with *Enterococcus* and *Bifidobacterium*.[72] These bacteria have been suggested to have anti-obesity effects.[33] Contrastingly, intake of high quantities of proteins from animal origin and fats such as n–3 PUFAs and MUFAs was positively correlated with *Gemella* and *Streptococcus*.[72] The genus Gemella and the species *Streptococcus oligofermentans*, have been positively associated with the prevalence of obesity.[74] An association between the intake of vitamin C and *Staphylococcus*, and between total intake and the *Bifidobacterium* genus has been reported.[71] Other researchers have shown that some phenolic compounds have the ability of promoting the growth of specific bacterial taxa, such as the *Lactobacillus* and *Bifidobacterium*genera.[75] However, further research needs to be conducted to establish a clear relation between the maternal diet and the milk microbiota given that this has a significant impact on the development of infant gut microbiota which contributes to infant health outcomes such as obesity in the short and long term.

Concluding comments and recommendations

This review investigated the impact of BM and its potentially bioactive compounds on the modulation of the infant gut microbiome and its influences on BW and the potential link

to obesity later in life. BM compounds in particular HMOs, probiotics, MFGM and adiponectin have been proposed to actively influence infant growth and BW by modulating the microbial diversity and composition of the gut. Additionally, MFGM in BM may promote a healthy infant BW by reducing the risk of metabolic endotoxemia through encouraging gut colonisation by beneficial bacteria. Probiotics have been hypothesised to have the potential of inhibiting fat accumulation, inflammation and the onset of obesity. HMOs were shown to play a significant role in the modulation of the gut microbiota and a concomitant impact infant growth. There was however, no strong correlation between BM components such as leptin, GLP-1, PYY and infants' BW. It is worth noting that the presence of confounding factors such as maternal diet, the timing and duration of breast feeding, in addition to the type of birth all have the potential of creating a spurious effect between breast milk and obesity later in life. Thus, further research needs to be conducted to establish a clear relation between the maternal diet and the milk microbiota as this has a significant impact on the development of infant gut microbiota which contributes to infant health outcomes such as obesity in the short and long term. Furthermore, additional studies with adequate and likely longer follows ups are needed in order to elucidate the link between breast milk, maternal diet, and the impact on the gut

microbiome composition Understanding how and which human milk compounds beneficially impact on an infants' BW is crucial to understand and close the gap between BM and FM and thus, potentially help in the development preventative public health strategies advocating breastfeeding as an effective approach in reducing risk of developing obesity in adult life.

Ethics Statement:

Given that this research uses publicly accessible documents as evidence, ethics approval was not required.

Conflict of interest:

During the process of drafting of the manuscript Porro Margherita started working at Mondelēz International. However, the authors declare that ideas and perspectives of this paper were not in any way influenced by Mondelēz. Additionally, the authors did not receive any financial contribution from Mondelēz for this research.

Kundrotaite Elena, Mellor Duane, and Munialo Claire declare that they have no conflict of interest.

Authors' contribution:

The research idea was conceived by CM and MP. MP, EK, DM, and CM jointly drafted, edited and revised the manuscript.

Competing Interests:

The authors declare no competing interests.

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Figure Legends

Figure 1. A schematic representation of the proposed links between maternal diet, maternal gut, human milk, and infant gut microbiomes. (1). Maternal diet can affect the composition of the gut microbiome. (2) Some bacteria are suggested to be transferred from maternal gut to

milk via an entero-mammary pathway. Some bacterial metabolites are transferred to and acting as prebiotics in the milk (3) Human milk can shape the infant gut microbiome via direct transfer of bacteria and exposure to microbiome-modulating factors such as SCFAs, and HMOs. It is worth noting that both bacteria and microbiome-modulating factors are produced by the mother and via maternal gut microbiota. (4) Maternal diet has been associated with the human milk microbiome. (5) Some studies support the association between maternal diet during pregnancy and the infant gut microbiome. (6) Maternal gut microbes may be transferred to the infant gut independent of breastfeeding. (7, 8) A Sharing of bacteria between mother and infant may occur as a result of a shared environment. Even though there may be an existence of shared taxa in infant and mother, this may not necessarily indicate a vertical transfer. This figure has been adapted from the work of Sindi et al 2021.[52]

Table 1: A summary of studies examining the potentially functional compounds found in breastmilk in animal models and their impact on growth parameters in infants

AUTHOR AND YEAR	STUDY SPECIES AND SAMPLE SIZE	INTERVENTION AND COMPARATOR	OUTCOME
Berding et al. 2016[15]	24 Two-day-old male piglets At 31 days, piglets were euthanised, and both Intestinal and faecal samples were collected for the microbiome analyses.	For 30 days, piglets were fed TEST formula milk with polydextrose (PDX) (1,2g/100 g diet), galactooligosaccharides (GOS) (3.5 g/100g diet), bovine lactoferrin (LF) (0.3 g/100 g diet) and milk fat globule membrane (MFGM) (2.5 g/100 g diet) or regular formula (CONTROL formula). Anthropometry data were taken during the dietary treatment.	From day 17 to day 31, pigs receiving the TEST diet weighed more than piglets on the control diet ($P<0.05$). At the end of the trial, TEST piglets weighed more (9.15 ± 0.27Kg) than the CONTROL group (8.47 ± 0.24) ($P=0.005$). Bacterial communities differed between TEST and CONTROL piglets in the ascending colon (AC) ($P=0.001$) and faeces ($P=0.047$), but not in the ileum ($P=0.679$). In the AC, TEST piglets had a lower abundance of *Mogibacterium, Collinesella, Klebsiella, Escherichia/Shigella, Eubacterium* and *Roseburia* than the CONTROL. TEST piglets had a greater count of *Parabacteroides, Clostridium IV, Lutispora* and *Sutterella.*
Gong et al. 2020[16]	Sprague Dawley rat pups	From postnatal day 8, rat pups were breastfed (BF) (n=16) or received regular infant formula (IF) (n=16) or infant formula containing a low dose of 260 mg Kg^{-1} body weight (BW) MFGM (IF + ML group n =16), a medium dose of 520 mg Kg^{-1} BW MFGM (IF + MH group, n=16), or a high dose of 1040 mg Kg^{-1}BW MFGM (IF + MH group, n=16). BW was recorded daily, and gut bacterial populations were analysed.	MFGM supplementation in IF reversed anthropometry trends as shown by elevated body weight compared with IF rat pups. The BF group had the greatest BW (g) (44.45 ± 0.42) followed by IF + MM (39 ± 0.25), IF +MH (38. 18 ± 0.62) and IF + ML rat pups (35.95 ± 0.24). IF rat pups had the lowest BW (34.53 ± 0.27). As a result, IF + MM rat pups had the most similar BW trend to BF rat pups among all feeding groups ($P < 0.05$). Additionally, MFGM supplementation in IF reversed and therefore increased the diversity of the intestinal flora in IF rat pups. Compared with the IF group, the intestinal flora of MFGM-supplemented rat pups was more like BF pups.

Table 2: A summary of studies examining the potentially functional compounds found in breastmilk in human studies and their impact on growth parameters in infants

AUTHOR AND YEAR	POPULATION	STUDY DESIGN AND MEASURES	OUTCOMES AND OBSERVATIONS
Plows et al., 2020[24]	Mother-infant dyads (1-month, n = 157; 6-months, n = 69).	BM samples were screened for 19 HMOs, and eating behaviour was assessed using the Baby Eating Behaviour Questionnaire (BEBQ)	At 1 month, difucosyllacto-N-tetrose (DFLNT) and disialyl-LNT (DSLNT) were negatively associated with food responsiveness in secretors only, whereas Lacto-N-neotetraose (LNnT) was negatively associated with responsiveness to food in the total sample. At 6 months, disialyllacto-N-hexaose, fucosyllacto-N-hexaose (FLNH), Lacto-N-hexaose (LNH6), and sialyllacto-N-tetraose c (LSTc) were positively associated with food responsiveness in both the total sample and secretors only.
Pekmez. et al., 2019[23]	Mother-infants' dyads with high weight gain (HW) (n=11) and normal weight gain (NW) (n=15) at 5 and 9 months of age.	BM and faecal samples were collecte for faecal SCFAs and HMOs	At 5 months of age, HW-group BM had lower levels of α-linolenic acid, oleic acid, 3-oxohexadecanoic acid, lysophosphatidylethanolamine (LPE) (P-16:0), lysophosphatidylcholine (LPC) (16:0), LPC (18:0), phosphatidylcholine (PC) (36:2). Transitioning from breastfeeding to complementary feeding resulted in a concurrent increase in the faecal SCFA concentrations. At 9 months, the NW group had a higher faecal butyrate concentration. Faecal-branched SCFAs were positively associated with: - (i) the levels of phospholipids in the BM, ii) the free-fatty acid levels, (iii) the diversity of HMO, sialylated-HMOs, 6'-sialyllactose, and disialyl-Lacto-N-hexaose.
Larsson et al., 2019[19]	13 high weight-gain (HW) and 17	HMO composition	Positive association between the total HMO concentrations HMO-bound fucose with both fat mass index (FMI) at 5 months. From 0 to 5

	normal weight-gain (NW) breastfed infants		months, a positive association was observed between HMO concentrations and the total HMO-bound fucose and weight velocity. Lacto-N-neotetraose was lower in the HW group and negatively associated with the height-for-age z-scores, the weight velocity from 0 to 5 months and the FMI. There was a negative association between the maternal BMI and 6'-sialyllactose and sialyl-Lacto-N-tetraose (LSTb) and a positive association between maternal BMI and 2'-FL, total HMO and total HMO-bound fucose at 5 months.
Alderete et al. 2015[17]	25 mother-infant dyads	BM and infant measures were taken at 1 and 6 months of infant age. HMO composition was analysed	Greater evenness and diversity of HMOs were correlated with lower fat mass %. A 0.40 kg decrease (P = 0.03) in infant weight with each 1 μg/mL increase in lacto-N-fucopentaose (LNFPI) at 1 month. At 6 months, a 1.11-kg lower weight (P = 0.03), 0.79-g lower fat mass (P = 0.02), and 0.85-g lower lean mass (P = 0.01) were observed with each 1μg/mL increase in LNFPI. Conversely, at 6 months, LNFPII and disialyl-lacto-N-tetraose were associated with a 0.42-g (P = 0.02) and a 1.92-g (P = 0.02) greater fat mass, respectively. Each 1-μg/mL increase in lacto-N-neotetraoseand fucosyl-disialyl-lacto-N-hexaose was associated with 0.03% lower (P < 0.01) and 0.04% higher (P = 0.03) body fat, respectively.
Sprenger et al. 2017[25]	50 mother-infant pairs (25 female & 25 males)	BM samples were collected at 1, 2 and 4 months postpartum. Five HMOs (2'FL, LNT, LNnT, 3'SL and 6'SL) were quantified. 2'FL was measured as representative for the FUT2 dependent HMOs as a	Up to 4 months, no differences in body length, weight, body mass index (BMI) were observed in infants breastfed by mothers with low 2'FL concentrations (median concentration between 15 and 10mg/L) and or with high 2'FL concentrations (median concentration of 2053 mg/L at 1 month, 1652 mg/L at 2 months and 1306 mg/L at 4 months).

		representative of the sialylated HMOs, 3'SL and 6'SL. LNT and LNnt were measured HMO core structures (sialic acid or fucose). Mother-infant pairs were grouped into low and high 2'FL (based on the measured milk 2' FL concentrations at 30 days of lactation from a representative milk sample). Infant's measures taken at birth, 1, 2 and 4 months.	
Davis et al 2017[18]	33 Gambian mother-infants' pairs	BM and infant growth parameters were taken at 4, 16, 20 weeks of age. Infant faecal samples were collected to study the associations between HMOs and infant microbiota.	At 20 weeks postpartum, 3'SL was positively associated with infants Weight-for-age Z scores (WAZ) while sialyllacto-N-neotetraose (LSTc) was negatively correlated. The abundance of Prevotella and *Bifidobacterium infantis* (*B. infantis*) was negatively associated with LSTc, but B. Infantis was positively associated with Lacto-N-neotetraose (LNnT). Lactobacillus was positively correlated with total fucosylation and Lacto-*N*-fucopentaose I (LNFP I) and Lacto-*N*-fucopentaose III(LNFP III). *Coriobacteriaceae* and *Megasphaera* were inversely correlated with total sialylation, fucosylated sialylated and Lacto-*N*-tetraose (LNT). Campylobacter was negatively correlated with LNT. Streptococcus was positively correlated with total sialylation, fucosylated, and LSTC, while

			Parabacteroides were positively correlated with monofucosyllacto-*N*-hexaose I (MFLNH I), monofucosyllacto-*N*-hexaose III (MFLNH III) and isomer III fucosyl-paralacto-*N*-hexaose (IFLNH III).
Pastor-Villaescusa et al, 2020[22]	291 mother-infants' pairs	16 weeks, the LC40 group (n=139) received 1 capsule /day containing 3x109 cfu Lc40; the control group (n=152) received 1 placebo (maltodextrin) capsule/day	A positive and significant correlation was observed for the Staphylococcus load between BM and infant faeces in the control group. The Lc40 group, infants whose mothers had higher values of Lactobacillus in their BM had significantly higher weight z-score.
Laursen et al., 2020[21]	30 exclusively breastfed infants	The role of the infant gut microbiota in excessive infant weight gain during breastfeeding was investigated	Abundance of gut Enterococcus and BM associated with reduced in excessive weight gain infants and their mothers BM compared with controls. An inverse correlation between Enterococcus abundance and age/gender-adjusted body weight, waist circumference and BMI, levels of plasma leptin and body fat.
Laursen et al., 2016[20]	114(SKOT I) & 113 (SKOT II) Danish children	Waist circumference, length-weight, and subscapularis skinfold thickness were measured at 9 and 18 months. Breastfeeding practices recorded.	Neither microbial diversity nor specific taxon abundance was influenced by maternal obesity. Instead, the main determinants of gut microbiota development were breastfeeding duration and composition of the complementary diet.