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Maternal modifiers of the infant gut microbiota - metabolic consequences

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Abstract

Transmission of metabolic diseases from mother to child is multifactorial and includes genetic, epigenetic, and environmental influences. Evidence in rodents, humans and non-human primates support the scientific premise that exposure to maternal obesity or high-fat diet during pregnancy creates a long-lasting metabolic signature on the infant innate immune system and the juvenile microbiota which predisposes the offspring to obesity and metabolic diseases. In neonates, gastrointestinal microbes introduced through the mother are noted for their ability to serve as direct inducers/regulators of the infant immune system. Neonates have a limited capacity to initiate an immune response. Thus, disruption of microbial colonization during the early neonatal period results in disrupted postnatal immune responses that highlight the neonatal period as a critical developmental window. Although the mechanisms are poorly understood, increasing evidence suggests that maternal obesity or poor diet influences the development and modulation of the infant liver and other end-organs through direct communication via the portal system, metabolite production, alterations in gut barrier integrity, and the hematopoietic immune cell axis. This review will focus on how maternal obesity and dietary intake influence the composition of the infant gut microbiota and how an imbalance or maladaptation in the microbiota, including changes in early pioneering microbes, might contribute to the programming of offspring metabolism with special emphasis on mechanisms that promote chronic inflammation in the liver. Comprehension of these pathways and mechanisms will elucidate our understanding of developmental programming, and may expand the avenue of opportunities for novel therapeutics.

Keywords

Obesity; Metabolism; Pregnancy; Neonatal; NAFLD

Introduction

Nearly two-thirds of American women of childbearing age are overweight or obese and greater than half these women, once pregnant, have excess gestational weight gain (GWG) (Deputy *et al.* 2015; Nicklas and Barbour 2015). Alterations in maternal microbiota

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Declaration of interest

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composition, associated with both diet and obesity status, have been widely reported (Collado *et al.* 2008; Collado *et al.* 2012; Paul *et al.* 2016; Santacruz *et al.* 2010). Disruption of the mother's gut microbiota can influence early pioneering bacteria in the newborn and may be a prime candidate for intergenerational transmission of metabolic disease risk given the considerable influence these microbes have on gut function and immune system development. The newborn gut begins as an aerobic environment, allowing only certain microbes to temporarily take up residence, followed by dramatic fluctuations in microbe composition with introduction of breast milk and solid foods (Voreades *et al.* 2014). Recent studies have proposed that colonization of the gastrointestinal tract begins *in utero* (Aagaard *et al.* 2014; Collado *et al.* 2016; Jimenez *et al.* 2008); however, this idea remains controversial, as discussed in detail by Perez-Muñoz, *et al.* (Perez-Muñoz *et al.* 2017). Regardless, the first major seeding of the gut microbiota is thought to occur during the birthing process following exposure to bacteria residing in the mother's vaginal canal and gut during vaginal birth or the mother's skin during a cesarean section (Dominguez-Bello *et al.* 2010). The mother's influence on neonatal acquisition of gut microbes continues by direct contact with the mother's skin and through breastfeeding, which provides critical nutrients to the colonizing bacteria and is a source of new bacteria. The diversity and density of bacteria continue to expand in response to new environmental exposures throughout the first few years of life until reaching a stable adult-like microbiota.

Several essential functions for human health develop in parallel with gut microbe expansion, including vitamin biosynthesis, energy extraction from the diet, gut barrier function, and immune system maturation/education. The immune system of neonates is immature and requires the exposure of gut bacteria to develop properly. Depending on the health of the mother, maternal microbe communities may already be imbalanced when passed on to the infant. In children, metabolic diseases, including obesity, insulin resistance and nonalcoholic fatty liver disease (NAFLD), along with other related immune-based diseases, are associated with alterations in infant gut microbe composition (Fig 1); however, the mechanisms remain unclear. This review will focus on maternal obesity, excessive GWG, and high-fat diet consumption during pregnancy and their impact on the microbiota of both mother and infant, including their links to early gut colonization and innate immunity in the infants that drive an increased risk for common metabolic diseases.

Development of the infant microbiota and immune tolerance

The gut is the most densely populated of all the sites of microbial colonization, containing over 10 trillion bacteria cells, represented by more than 1000 different bacterial species. Mammals have evolved a mutual relationship with gut microbes, commonly referred to as "commensal". Colonization of the infant gut by these commensals during the first few years of life is a time of significant fluctuation and maturation. Early alterations in the normal succession of the gut microbiota may have an impact on future colonization (Eggesbø *et al.* 2011), which could have lasting health consequences.

Commensal colonization progresses through a choreographed succession of bacterial species, and evolves rapidly during the first months of life (Del Chierico *et al.* 2015; Hill *et al.* 2017). Systemic responses to commensal microbiota are essential for the maintenance of

beneficial host-microbiota interactions. For example, maternally acquired IgA and IgG in neonatal mice leads to dampened T cell-dependent immune responses against commensal bacteria (Koch *et al.* 2016). Additionally, systemic IgG responses to Gram-negative bacteria, acquired early and over the course of life, were shown to provide cross-protection against Gram-negative pathogens, such as *Escherichia coli* and *Salmonella*, in mice (Zeng *et al.* 2016). These observations reinforce the concept that microbial composition and the timing of host-commensal interactions provide the foundation for balanced immunity in the intestine.

Under healthy conditions, vaginally-delivered infants born to normal-weight mothers are initially colonized by mostly facultative anaerobic bacteria including *Staphylococcus*, *Streptococcus*, *E. coli*, and *Enterobacteria*. These anaerobes make the habitat suitable for colonization by strict anaerobes including *Bacteroides*, *Bifidobacterium*, and *Clostridium* (Palmer *et al.* 2007; Penders *et al.* 2006), by consuming oxygen, altering the pH, lowering the redox potential, and producing carbon dioxide and nutrients. Jakobsson, et al. (Jakobsson *et al.* 2014) reported a similar succession of colonization, including an initial blooming of Proteobacteria that gradually declined from 1 week to 24 months, Firmicutes that expanded from 3 months onward and a peak of Actinobacteria at 3 months. This pattern of colonization was similar in infants born by cesarean section, except the notably higher proportion of Bacteroidetes in vaginally-delivered infants during the first 12 months of life. *E. coli* is a member of the Proteobacteria phylum and is the most abundant facultative anaerobe in the mammalian gut microbiota, but under homeostatic conditions, this species represents only a minor fraction of the ecosystem. Nevertheless, Proteobacteria abundance may explain significant functional variability in the human gut microbiome (Bradley and Pollard 2017). Importantly, *E. coli* (like many other members of the family Enterobacteriaceae) has a short doubling time and a highly flexible metabolic capacity. These attributes make this bacterium highly adaptable and allow it to bloom in the presence of oxygen and nitrogen.

The importance of Proteobacteria to immune education in early life

An emerging paradigm, originally known as “The Restaurant Hypothesis” (Leatham-Jensen *et al.* 2012), posits that nutritional stress may alter the original colonizing bacteria, including *E. coli*; therefore, the signaling pathways controlled by microbial mediators may cause fine-tuning in the gut, potentially altering the immune system in early life. Several studies suggest that the critical immune mechanisms controlling the maintenance of homeostasis and tolerance to environmental exposures are determined by microbial-host interactions occurring during a narrow time frame contained within the earliest days of life (Koch *et al.* 2016; Zeng *et al.* 2016). The early colonization of Proteobacteria and its subsequent reduction in abundance over time is thought to be an important part of this process, whereby a disruption of this progression has been linked to an increased risk of neonatal diseases, particularly in pre-term infants (Neu 2015, 2016). The Proteobacteria bloom in the neonatal gut is likely under maternal control. Human milk oligosaccharides (HMOs) (De Leoz *et al.* 2015) and secretory IgA production (Mirpuri *et al.* 2014) are involved in the selective suppression of Proteobacteria during establishment of the early gut flora.

Proteobacteria are believed to be important contributors to inflammation associated with metabolic disease in adults, and their role in infant immunity is critical for early priming of the innate and adaptive immune system. A primary structural component of Gram-negative bacteria residing in the gut is the endotoxin lipopolysaccharide (LPS). Translocation of LPS from the gut into the systemic circulation has been widely reported in metabolic disease (Cani *et al.* 2007; Lassenius *et al.* 2011; Ruiz *et al.* 2007). A common theme that has recently emerged is that variability in the lipid A component of LPS can tip the balance of innate immune responses towards homeostatic tolerance or pro-inflammatory signaling, affecting adaptive immune responses that either alter or inhibit protective signaling normally induced by toll-like receptors (TLRs). Lipid A, the biologically active moiety of LPS, can be expressed in variant forms by many human pathogens, allowing for evasion of the host innate immune system and establishment of a chronic infection. Strikingly, several of these host-adapted Gram-negative bacteria that express immune-evasive lipid A are associated with increased risk of autoimmune disease (Vatanen *et al.* 2016), type I diabetes, atherosclerosis (Erridge *et al.* 2007b; Slocum *et al.* 2014), and allergic disorders in childhood and adulthood (Grönlund *et al.* 2000; Ogra and Welliver 2008). A role of lipid A variants is to allow a bacterium to evade TLR4 and promote chronic inflammation and these variants appear to act through dysregulation of both innate and adaptive immune responses (Kramer and Genco 2017).

While a close relationship exists between microbial flora and the intestinal epithelium, the mechanisms whereby the mucosal surface senses the presence of colonizing microbial organisms and allows for innate immune receptor-mediated responses is largely unknown (Hooper *et al.* 1999). Recognition of microbial structures by mammalian cells occurs through various transmembrane receptor molecules that are part of the innate immune system. TLRs recognize conserved microbial structures, such as LPS, mediate cellular activation, and thereby provide the costimulatory signal required for an efficient immune response (Medzhitov 2001). Initial exposure to bacteria and LPS in early infancy has been shown to contribute to the development of the immune system by educating it on how to appropriately respond to bacteria and their components. Different bacterial species have variations in their LPS structure that impact their ability to elicit an innate immune response (Vatanen *et al.* 2016; Whitfield and Trent 2014).

The immunological phenomenon wherein prior exposure to TLR ligands renders macrophages refractory to LPS-elicited pro-inflammatory cytokine secretion is known as endotoxin tolerance (Biswas *et al.* 2007). Endotoxin tolerance is classically induced by exposure to low levels of LPS, thereby rendering the cells “tolerant” to subsequent endotoxin challenge, characterized functionally by a marked inhibition of inflammatory cytokine (e.g. TNF- α , IL-1, and IL-6) production upon re-challenge with endotoxin (Erroi *et al.* 1993; Granowitz *et al.* 1993). This immune-regulatory process of induction is also associated with a unique metabolic phenotype in macrophages that facilitates persistent improvements in antimicrobial function (phagocytosis) against Gram-negative bacteria (Coveney *et al.* 2015; Fensterheim *et al.* 2017). Immediately following TLR stimulation, macrophages shift their metabolic profile to one that favors glycolysis over oxidative metabolism (Everts *et al.* 2014) promoting a pro-inflammatory phenotype. In contrast, anti-inflammatory macrophages exhibit a metabolic program of augmented oxidative

phosphorylation (Kelly and O'Neill 2015). The lack of appropriate alterations in early immune education may affect the overall functionality of the microbiota and macrophages thereby altering inflammation and key pathogens that can drive shifts in the community composition into dysbiosis later in life.

We have recently shown in a prospective study on vaginally-delivered, exclusively breastfed infants with no neonatal or postnatal exposure to antibiotics that neonates born to mothers with obesity showed a significant 50% reduction in Gammaproteobacteria at 2 weeks of age compared with infants of normal-weight mothers (Lemas *et al.* 2016). A striking relative depletion in Proteobacteria species was recently found in 2-day-old neonates delivered vaginally, but not by cesarean section, to overweight/obese mothers (Mueller *et al.* 2016), suggesting the differences in relative abundance of Gram-negative bacteria may stem from vertical transmission of the maternal microbiota. It is also important to note that maternal overweight/obesity could be due to a mix of environmental as well as genetic factors not accounted for, such as excess GWG or poor diet, both of which likely has its own effect on microbial community structure. Based on studies in mice (Mirpuri *et al.* 2014), induction of a Gammaproteobacteria-specific IgA response partially regulated the transition from a neonatal to a mature microbiota. Experiments in germ-free mice showed that colonization with microbes from mice lacking IgA had persistent increased colonization with Gammaproteobacteria that resulted in sustained intestinal inflammation and increased susceptibility to neonatal and adult models of intestinal injury. Thus, the effects of a relative depletion of intestinal Proteobacteria species in neonates of mothers with obesity could cause persistent alterations in immune development and increase an infant's risk of developing inflammatory and metabolic diseases, but the literature on this topic in full-term human neonates is scarce.

One theory for why mothers with obesity have infants with reduced Proteobacteria and potentially more inflammation could be as simple as micronutrient deficiency. Although not universal, mothers with obesity and those with excess GWG are iron-deficient (Dosch *et al.* 2016; Jones *et al.* 2016), as well as their infants. A wide range of host immune responses are intricately linked with both systemic and cellular iron homeostasis. Perturbations to the labile iron pool have been linked with modulating various forms of innate and adaptive immune functions including proliferation, differentiation and secretion of inflammatory mediators (Ellermann and Arthur 2017). Processes associated with inflammation, including cytokine production and engagement of TLRs by microbial ligands, stimulate the production of the hormone hepcidin, which in turn modulates systemic iron homeostasis by limiting dietary iron absorption and increasing intracellular retention of iron (Drakesmith and Prentice 2012; Nemeth *et al.* 2004a; Nemeth *et al.* 2004b). Given that bacteria can secrete small molecules known as siderophores that can solubilize iron for host cells (Deriu *et al.* 2013), the opportunity is present for resident bacteria to modulate host immune responses at the mucosal interface. Iron has also been shown to modulate butyrate-producing bacteria (Tang *et al.* 2016), as well as pathogenic Proteobacteria (Kamada *et al.* 2013). Whether iron deficiency has a direct effect on early microbiota colonization and inflammation is a hypothesis worthy of further study.

Maternal gestational weight status and dietary intake impacts the development of the infant microbiota

The mother has a direct role in initially colonizing the infant microbiota as is evidenced by the fact that infants born vaginally have different microbiota compositions than infants born by cesarean sections (Dominguez-Bello *et al.* 2010). While it is unclear whether these early differences in microbiota composition affect the subsequent commensal colonization into childhood and beyond, it is clear the mother's microbes do contribute to the colonization process. The gut microbiota has been shown to be altered with advancing gestation in normal-weight mothers, and when these microbes were transferred into germ-free mice, the mice receiving microbes from mothers in the 3rd trimester showed increased weight gain, inflammation, and insulin resistance (Koren *et al.* 2012). It should come as no surprise that conditions like obesity, weight gain, and diet also alter the maternal microbiota composition during pregnancy, but whether this has a significant impact on metabolic disease risk is still under investigation.

To date there are only a few studies that have explored the impact of maternal obesity and diet on maternal and infant microbiota, but the existing data suggests there is a significant impact. Collado, et al. (Collado *et al.* 2008) found that women that were obese prior to pregnancy had significantly different gut microbiota compositions compared with normal-weight pregnant women during both the 1st and 3rd trimesters of pregnancy. Furthermore, women that gained excessive gestational weight regardless of pre-pregnancy BMI had significant differences in their microbiota composition compared with women who had normal weight gain. Specifically, *Bacteroides* and *Staphylococcus* were found to be significantly higher in obese women and *Bacteroides* species were elevated in all women with excessive GWG. Santacruz, et al. (Santacruz *et al.* 2010) also found a difference in gut microbiota composition between normal-weight and overweight women. Overweight women were found to have significantly more *Staphylococcus*, *Enterobacteriaceae* and *E. coli* and fewer *Bifidobacterium* and *Bacteroides* compared with normal-weight pregnant women. They also reported that women with excessive GWG had significantly more *E. coli* and fewer *Bifidobacterium* and *Akkermansia muciniphila* than in women with normal weight gain during pregnancy. While there are some inconsistencies in the results of these studies, it is important to note that the samples were taken at different times during pregnancy and that one study compared normal-weight women to women with obesity and the other compared normal-weight and overweight women, which could account for the different findings. While the exact changes that occur in the microbiota of pregnant women with obesity are unclear, the important observation is that their microbiota is different from normal-weight pregnant women and that these differences (whether due to diet or body habitus) could influence the microbial colonization of the infant, as noted earlier. Collado, et al. (Collado *et al.* 2010) compared the microbiota of infants at 1 and 6 months of age that were born to overweight mothers (based on pre-pregnancy BMI) versus mothers that were normal weight. Infants born to overweight mothers were found to have increased *Bacteroides* and *Staphylococcus* at 1 and 6 months of age. This observation coincides with what this group showed in mothers during pregnancy (Collado *et al.* 2008), providing evidence for the mother's role in directing the early infant microbiota composition. Maternal

obesity has also been associated with long term changes in the offspring microbiota. Galley, et al. (Galley *et al.* 2014) found that toddlers born to mothers with obesity had significantly elevated levels of *Oscillibacter*, *Parabacteroides*, and an unclassified Bacteroidales genus, and reduced levels of *Eubacterium* and *Blautia* compared with toddlers born to normal-weight mothers; however, these studies did not control for mode of delivery, antibiotic use, or breastfeeding exposure.

Maternal diet has also been shown to have a significant impact on the microbiota composition of the offspring. Recently, Chu, et al. (Chu *et al.* 2016) showed that a maternal high-fat diet, estimated from retrospective surveys, resulted in a depletion of *Bacteroides* and an enrichment of *Enterococcus* in the meconium and a trend for reduction of *Bacteroides* at 6 weeks of age. Evidence of the impact on maternal diet has also been reported in animal models including both rodent and non-human primate models. In a study by Myles, et al. (Myles *et al.* 2013), feeding pregnant mouse dams a Western-style diet resulted in an altered microbiota composition in the offspring including an increase in Clostridiales (a class of Firmicutes), despite being weaned onto a control diet. In addition to having an altered microbiota composition, these offspring also had worse outcomes in models of infection, autoimmunity, and allergic sensitization. Importantly, in non-human primates, infants born to mothers fed a high-fat diet during gestation and lactation (whether obese or not) showed significant intestinal dysbiosis that was not completely corrected by switching to a control diet after weaning. This has important clinical relevance as the early colonized bacteria, driven by maternal diet, may have a long-lasting effect on the commensal microbe population in offspring, therefore setting the stage for increased risk for immunologic and metabolic disease patterning.

The important role of breast milk in colonization of the infant gut microbiota

The impact of the mother's microbiota on the colonization of the infant gut continues after initial colonization at birth through breastfeeding. Breast milk is a source of live bacteria and contains energy sources for colonizing bacteria. Breast milk has also been shown to play a role in preventing the colonization of pathogenic bacteria (Jantscher-Krenn *et al.* 2012). Assessment of the bacterial composition of breast milk in healthy women has revealed the presence of a wide variety of bacteria, including *Staphylococcus*, *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, and *E. coli* (Heikkilä and Saris 2003; Martín *et al.* 2007). Conversely, breast milk from mothers with obesity has been shown to harbor a different and less diverse bacterial community than that of normal-weight subjects, including higher levels of *Staphylococcus* and *A. muciniphila* and lower levels of *Bifidobacterium* (Cabrera-Rubio *et al.* 2012; Collado *et al.* 2012), and a different composition of hormones, cytokines, and oligosaccharides (Andreas *et al.* 2014). The source of bacteria in breast milk is unclear, but there is evidence suggesting bacteria residing in the gut might be a source of the breast milk populations (Latuga *et al.* 2014; Rodríguez 2014).

HMOs are an important component of breast milk and given the fact that they are indigestible to the human host, they are now believed to exist for the primary purpose of

providing nutrients to the microbes colonizing the infant gut. There are over 200 known HMO molecules that are present in human breast milk which vary across individuals (Ninonuevo *et al.* 2006). Recently, differences in HMO composition in mother's milk have been associated with infant growth and body composition (Alderete *et al.* 2015).

Breastfeeding compared to formula feeding is thought to provide significant health benefits to an infant, specifically reducing the risk of obesity (Binns *et al.* 2016; Dieterich *et al.* 2013), where the risk is inversely related to the duration (Ip *et al.* 2009). Given the microbial and HMO differences found in the breast milk of obese women compared with normal-weight women, this benefit may not be universal, but more research is needed to determine if the benefits outweigh the risks. Support for this concern is evidenced by the fact that antibiotic use in infants born to normal-weight mothers increases the risk of obesity, whereas antibiotic use in infants born to mothers with obesity reduces the risk of obesity (Ajslev *et al.* 2011). This indirectly suggests that it might be advantageous to alter the composition of the gut microbiota in infants born to mothers with obesity.

Obesity and the programming of hematopoietic immune cells

The microbiota has been implicated in altering immune system function via changes in hematopoiesis. Dietary changes can have major effects on microbial composition and release of LPS that provokes inflammation, insulin resistance, and may even program bone marrow-derived stem cells (Giusti *et al.* 2017). The significant increase in common myeloid progenitor cells derived from the bone marrow in diet-induced obese mice is thought to be the result of an altered microbiota composition (Luo *et al.* 2015). Indeed, recent evidence has emerged to indicate that innate immune memory can be transferred via hematopoietic stem and progenitor cells that alter the function of their differentiated progeny (Ng *et al.* 2013), depending upon the composition of the microbiota (Burgess *et al.* 2014). Thevaranjan, *et al.* (Thevaranjan *et al.* 2017) showed that old mice had a distinct microbiota composition compared with young mice. Bone marrow-derived macrophages and whole blood from germ-free mice colonized with gut microbiota from the old mice produced increased levels of inflammatory cytokines following stimulation by LPS compared with young mice. Singer, *et al.* (Singer *et al.* 2014) found that high-fat diet increases the production of bone marrow-derived dendritic cells and peritoneal macrophages from obese mice and they produce more inflammatory cytokines including TNF- α and IL-6 following LPS stimulation than lean mice; their findings suggest that this pro-inflammatory profile persists after the obesogenic diet is removed.

Commensal bacteria at the crossroad between inflammation and childhood NAFLD

Maternal obesity and diabetes are among the most powerful predictors of childhood obesity and other adverse health outcomes such as NAFLD, that affects up to 34% of obese children (Anderson *et al.* 2015). A particularly alarming statistic is the prevalence of obesity in children, affecting 10% of infants and toddlers and 17% of children and adolescents in the United States (Ogden *et al.* 2012). With the rise of obesity, it is predicted that NAFLD will be the most common etiology for liver transplantation in the 21st century (Agopian *et al.*

2012). A cross-sectional study of 538 children with biopsy-proven NAFLD illustrated the critical importance of the *in utero* environment to development of pediatric NAFLD (Newton *et al.* 2017). They showed that children born with high or low birth weight had >2-fold significantly increased incidence of nonalcoholic steatohepatitis (NASH) or advanced fibrosis, respectively, compared with those of normal birth weight.

Evidence suggests that the transgenerational passage of the maternal obese phenotype with NAFLD, or developmental programming of the offspring, involves the innate immune system as well as metabolic perturbations by gut microbes (Wesolowski *et al.* 2017). Because early immune development is highly dependent on triggers provided by the microbiota (Ma *et al.* 2014), infants born to obese mothers might be exposed to products of gut dysbiosis that activate macrophages in the liver and bone marrow. However, the timing, mechanisms involved, and whether interventions that alter microbial colonization can prevent early innate immune system “programming” are unknown. Results from recent studies demonstrate that the maternal microbiota impels early postnatal innate immune development (Gomez de Agüero *et al.* 2016), while in adults the transmissibility of obesity and related disorders by gut bacteria is likely mediated specifically by cells of the innate immune system, a phenomenon that is observed in Rag1^{-/-} mice lacking all cells of the adaptive immune system (Bäckhed *et al.* 2004).

The source of inflammation in macrophages associated with NAFLD is incompletely understood; however, increased circulating levels of the bacterial-derived endotoxin LPS is believed to play a role. Alterations in microbial composition have been associated with increased gut permeability, resulting in translocation of LPS. Binding of LPS to TLRs triggers the release of cytokines and an associated inflammatory response. LPS will bind to LPS binding protein (LBP) and that complex will bind to CD14, located in inflammatory cells. Together LPS-LBP-CD14 activates TLR4, which is present in hepatocytes, adipocytes, Kupffer cells, the most abundant macrophage in the body, and hepatic stellate cells, triggering the release of inflammatory cytokines (i.e. TNF- α , IL-1 β , and IL-6). Furthermore, the translocation of LPS from the gut is not reserved to individuals with disease. Studies have shown the ingestion of meals containing fat increase systemic LPS concentrations in healthy individuals (Erridge *et al.* 2007a; Ghanim *et al.* 2009; Ghanim *et al.* 2010; Laugerette *et al.* 2011). Under normal circumstances the immune system has a normal inflammatory response to an acute endotoxin stimulus and once the toxin is neutralized and removed, the state of inflammation returns to baseline levels. If endotoxin exposure becomes chronic, a low-grade inflammatory state may be maintained, contributing to tissue damage and the development of metabolic diseases. Those microbes most successful at driving inflammatory disease processes have evolved to interfere with host gene expression and metabolism. Many of these pathogens persist inside the cells of the immune system. Miskinyte and Gordo (Miskinyte and Gordo 2013) examined how a commensal *E. coli* was able to develop the virulence needed to persist inside macrophages of the immune system. Working in mice, they found that when maintained *in vitro* under the selective pressure of host macrophages, commensal *E. coli* can evolve virulent clones that escape phagocytosis and macrophage killing *in vitro*, while increasing their pathogenicity *in vivo*.

A study by Cani, et al. (Cani *et al.* 2007) showed that an infusion of LPS for 4 weeks in mice led to increased weight gain and development of insulin resistance, similar to mice fed a high-fat diet, providing a causative role for gut microbiota in the development of metabolic disease. In our non-human primate model of maternal obesity, high-fat diet exposure increased fetal hepatic oxidative stress and apoptosis in the early 3rd trimester, perhaps priming the liver for later development of NASH (McCurdy *et al.* 2009). Furthermore, there is innate immune dysfunction and necro-inflammatory changes in juvenile offspring of high-fat diet-fed dams (Thorn *et al.* 2014). Importantly, these alterations persist even after weaning to a normal chow diet. Lastly, in this model, a maternal high-fat diet, regardless of maternal weight gain, leads to persistent gut dysbiosis in juvenile offspring switched to a healthy diet at weaning (Ma *et al.* 2014).

Emerging evidence suggests that the severity of NAFLD postnatally is strongly associated with gut dysbiosis (Boursier *et al.* 2016) and a shift in gut metabolic function, including production and utilization of short-chain fatty acids and bile acids. Given the early gut permeability engendered by a dysbiotic infant microbe community, it is tempting to speculate that certain bacteria and their outputs might trigger programming events in the liver, and in progenitor cells in the bone marrow as well (see discussion earlier). We recently found that pregnant mice fed a Western-style diet produced an early programming effect on the gut microbiota and on bone marrow-derived macrophage polarization in 3-week-old offspring, suggesting that shaping of the early gut microbiota and programming of macrophages during the early weaning period are crucial for development of NAFLD and fibrosis in adulthood (K R Jonscher & J E Friedman, unpublished observations). The early microbes and the microenvironment and mechanisms responsible for programming myeloid cells within the bone marrow and liver of obese offspring warrant further research.

Problems and future challenges in determining causation in early microbiota colonization

Early alterations in the infant microbiota composition are associated with the development of both metabolic and immune diseases, but causation is lacking. Models imploring the use of germ-free animals or antibiotics have been the primary methods for isolating and studying the impact of the microbiota on human health. Studies using germ-free animals have produced compelling evidence for the role of the microbiota in obesity development and education of the immune system (Ridaura *et al.* 2013; Turnbaugh *et al.* 2006; Weng and Walker 2013), improving our understanding of the contribution of the microbiota in disease progression. Unfortunately, these studies do not provide sufficient information about the role of the microbiota in the initiation of the disease, or whether an altered microbiota is a cause or just a consequence of disease. Another challenge that exists is that there is still significant controversy over what is considered a healthy or diseased microbiota. This controversy is highlighted in a recent review (Sze and Schloss 2016) evaluating studies that reported the microbiota in subjects with obesity, which suggests that we are still unable to conclusively define the composition of an obese microbiota. One reason it has been difficult to assign health benefits to a specific microbiota composition is because of the functional redundancy found across various bacterial groups (Moya and Ferrer 2016). Future studies are needed to

explore the impact of individual bacterial species, their interactions with each other and with their host, and how they contribute to disease development.

Long term studies are needed to determine if an altered microbiota composition alone is sufficient for disease development or whether a secondary stressor like diet is needed to accelerate disease. Identifying a programming effect due to early “obese microbes” that fine tunes the infant gut may require a second hit to reveal the causes for developmental programming of the immune and metabolic systems in early life. This has been done on a limited basis in studies showing that microbial composition dictates susceptibility to disease development in humans (Spencer *et al.* 2011) and animals (Le Roy *et al.* 2013) providing support for a causal relationship, but only in response to a secondary insult (i.e. diet). Ongoing studies are currently exploring the interaction between the microbiota composition and diet to better understand the impact of this interaction in disease development. Additionally, determining whether there are legacy costs of early alterations in microbial succession that are maintained long after the microbiota has adapted to solid foods and reached maturity and stability are also important to understand the full impact of an early alteration in the infant microbiota composition. As we continue to learn more about the importance of early bacterial colonization, we can develop strategies for interventions that could reduce the future incidence of a wide variety of metabolic and immune diseases.

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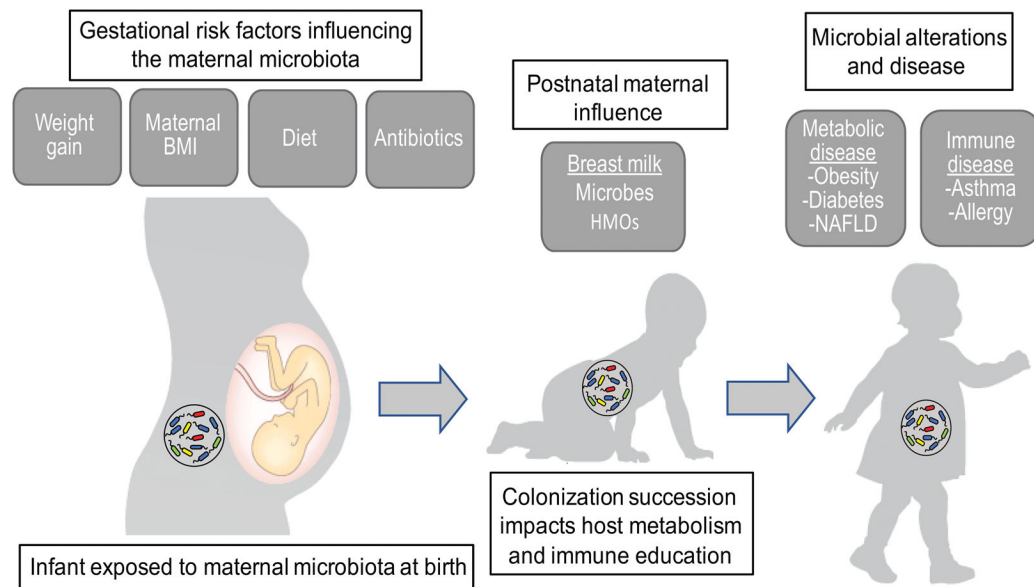


Figure 1.

Influential factors on the maternal and infant microbiota. Maternal factors significantly contribute to the initial colonization and succession of the infant gut microbiota. Alterations in this process may have long term health consequences related to host metabolism and immune education. HMOs, human milk oligosaccharides; NAFLD, nonalcoholic fatty liver disease.