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Themed Issue: The Gut Microbiome and Immunity: How it is Shaped by Early Life

REVIEW

The impact of early life gut colonization on metabolic and obesogenic outcomes: what have animal models shown us?

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The rise in the occurrence of obesity to epidemic proportions has made it a global concern. Great difficulty has been experienced in efforts to control this growing problem with lifestyle interventions. Thus, attention has been directed to understanding the events of one of the most critical periods of development, perinatal life. Early life adversity driven by maternal obesity has been associated with an increased risk of metabolic disease and obesity in the offspring later in life. Although a mechanistic link explaining the relationship between maternal and offspring obesity is still under investigation, the gut microbiota has come forth as a new factor that may play a role modulating metabolic function of both the mother and the offspring. Emerging evidence suggests that the gut microbiota plays a much larger role in mediating the risk of developing non-communicable disease, including obesity and metabolic dysfunction in adulthood. With the observation that the early life colonization of the neonatal and postnatal gut is mediated by the perinatal environment, the number of studies investigating early life gut microbial establishment continues to grow. This paper will review early life gut colonization in experimental animal models, concentrating on the role of the early life environment in offspring gut colonization and the ability of the gut microbiota to dictate risk of disease later in life.

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Introduction

The worldwide prevalence of obesity is now recognized to have reached epidemic proportions. The World Health Organization reports that at least 1.9 billion adults are overweight while 600 million are clinically obese.[1] Obesity is especially pronounced in young individuals, including children, and women of reproductive age.[2,3] Maternal obesity is associated with an increase in both maternal and fetal complications during pregnancy;[4] where maternal obesity not only impacts the health of the mother,[5,6] it also has short- and long-term ramifications for her offspring.[7]

It is now well established that disease risk strongly correlates with early life environment, and offspring responses to the postnatal environment are modulated by intrauterine adaptations.[8,9] The mechanistic signaling pathways linking maternal to offspring disease risk remain unclear. With the emergence of the gut microbiota as a factor influencing disease risk,[10,11] new concepts governing how the colonization of the infant gut occurs,[12–15] and the role of infant gut colonization in mediating the link between maternal and child obesity are evolving. In this paper, we review the current animal literature investigating gut colonization early in life, and the role of the gut microbiota in mediating obesity risk in the context of maternal overweight and obesity.

Early life nutritional programming of offspring obesity and metabolic dysfunction

Since the advent of the Developmental Origins of Health and Disease (DOHaD) hypothesis established through the early investigations of epidemiological data sets,[16–18] many animal models have consistently produced data to support the hypothesis that the intrauterine environment plays a central role in offspring metabolic compromise and obesity risk. Studies in both large animals, such as the sheep and pig, and small animals including the mouse, rat and guinea pig, have been instrumental in advancing our understanding of the relationship between early life adversity and the risk of developing disease later in life. In rodents, diet-induced maternal obesity results in offspring that are often growth restricted,[19,20] but not always,[21,22] that display postnatal catch-up growth,[19,22] exhibit hyperinsulinemia and hyperleptinemia, insulin resistance as well as defects in pancreatic signaling and insulin secretion,[19,23–26] and hepatic glucose and lipid handling dysfunctions.[20,27–29] These effects are often magnified when offspring are themselves fed a postweaning high fat diet, highlighting interactive effects between the pre- and postnatal environments.[19,21,22,30] These observations are similar to those observed in large animals, where maternal

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obesity induces impaired peripheral insulin signaling in offspring,[31] defects which appear present already during fetal life.[32,33] Although less numerous, studies in non-human primates are consistent with those done in small and large animal models, showing significant metabolic impairments in offspring born to obese/high fat fed mothers.[34–39] Despite great advances in our understanding of the molecular and physiological pathways that govern the relationship between our early life environment and long-term disease risk, the fundamental mechanisms underpinning the link between maternal obesity and childhood obesity are still unclear. As such new lines of investigation are constantly explored in an effort to identify targets for intervention. One such system is the role of the gut microbiota and the host–microbe relationship in mediating gut development, nutrient absorption and immune function.

The gut microbiota

Trillions of microbes reside within the human gut; collectively these microbes constitute our microbiota, and the genes they encode and the environment that they inhabit are known as our microbiome.[40] Coined as our 'forgotten organ', these commensal bacteria live symbiotically within the host, having coevolved with vertebrates over many millennia.[41] The intestinal commensals play an essential role in the maintenance of host health as they are responsible for the metabolism of indigestible polysaccharides, the production of essential nutrients, the regulation of fat storage[42] and protection from pathogenic bacterial colonization (for review, see Nieuwdorp *et al.*[43]). Commensal bacteria and their metabolites are essential for the normal development and function of the mammalian immune system, and the maintenance of gut epithelial barrier integrity.[44,45] Studies in germ-free mice have shown that the gut microbiota is essential for the growth and development of the gastrointestinal system.[46] These gnotobiotic mice have reduced intestinal weight and surface area and poor nutrient absorption,[47] as commensal bacteria are required for the breakdown of nutrients.[48] Perturbations in this ecosystem, including those associated with diet, have been linked with an increased risk of developing non-communicable disease including obesity (for review, see[49,50]). The most cited microbial dysbiosis that characterizes an obesogenic phenotype is a shift in the two most prominent phyla in the mammalian gut; *Firmicutes* and *Bacteroidetes.* There is currently no sound evidence characterizing the mechanisms that establish a microbial profile commiserate those observed in a lean 'healthy' phenotype *v.* those of an obese 'diseased' phenotype. Furthermore, there exists a large discrepancy in the literature as to which bacterial genera and species are associated with deleterious outcomes. What constitutes a 'healthy' microbiota from the time of gut establishment, to maintenance throughout life, fails to exist in the literature. Despite these gaps, however, emerging studies have begun to shed some light on host–bacterial relationships and their role in metabolic phenotype.

Early life gut colonization: how early is early?

The hypothesis that the fetus develops within a sterile intrauterine environment was originally proposed over 100 years ago by French pediatrician Tissier,[51] and since then has gone largely unchallenged, primarily due to the fact that many obstetric complications including preterm birth have been associated with intrauterine infection.[52] As such, it has long been proposed that under normal circumstances, the infant acquires a bacterial inoculation at the time of birth, where exposure to maternal fecal and vaginal microbes establishes the budding neonatal gut microbiota. Early animal studies supported this contention.[53] However, improvements in large-scale sequencing and computational analyses of metagenomics[54] have revealed alternative theories. Increasing evidence suggests that the developing fetal gut may be seeded before birth. Bacterial populations associated with the maternal gastrointestinal tract[55] have been isolated using both culture-dependant and independent techniques, from meconium,[55,56] fetal membranes[55] and from umbilical cord blood of healthy neonates.[57] Pioneering animal studies demonstrated that oral administration of pregnant mice with genetically labeled *Enterococcus fecium* resulted in the presence of this bacterium in fetal meconium after sterile C-section,[55] supporting the hypothesis that the fetus may be exposed to maternally gut-derived bacteria *in utero* and that this exposure may prime gut development. Thus, it is possible that the maternal microbiota provides the first fetal bacterial inoculum and then from birth, microbial diversity increases to converge to an adult-like profile. We understand little about the developing 'fetal microbiota'. However, factors including maternal gut bacterial load, bacterial composition, diet, genetics,[58] mode of delivery, antibiotic use[59,60] intestinal tract development,[61] bile acids, pH[59] and host–microbial interactions,[62] which influence microbial establishment have allowed researchers to begin to understand more about how our gut microbiota is established, and how it is maintained over the life course.

Factors influencing early life microbial colonization

Maternal bacterial translocation during pregnancy

The concept that maternal gut microbes may prime fetal immune development has recently gained interest in the current literature. Pioneering studies in mice show that during pregnancy and lactation there exists bacterial translocation to extra-intestinal tissues, where pregnant mice have gut-derived bacterial presence in mesenteric lymph nodes, and within 24 h after delivery, had viable bacteria in mammary tissue.[63] In contrast to pathologic conditions in which the translocation of microbes are mainly Gram-negative, penetrating species in pregnant and lactating mice included *Streptococcus*, *Lactobacillus* and *Bifidobacterium*, whose numbers subsided gradually over time.[63] Recent human data suggest that the placenta may harbor a microbiota,[12] and although not yet shown in animal studies, the maternal translocation of gut microbes to the placenta has biological plausibility.

Despite the fact that translocation through the gut epithelium is prohibited by a well-established barrier,[45] dendritic cells can paracellularly sample the gut lumen and its bacteria/bacterial components to facilitate bacterial transport to the periphery through lymph.[64,65] Whether pregnancy changes gut barrier function and/or dendritic sampling is unclear, however, data in mice show that a significant proportion of pregnant animals harbor bacteria in mesenteric lymph nodes during pregnancy compared with their non-pregnant counterparts.[63] Dendritic cell sampling may possibly explain bacterial translocation from the gut to peripheral sites such as the placenta, resulting in the presence of bacteria in fetal meconium.[55] These data are consistent with those derived from a non-human primate model where the bacterial species isolated from the monkey milk were found to be gut commensals.[66] These early studies support the notion that during pregnancy, bacterial translocation to peripheral sites can occur – indeed a recent review has highlighted the fact that maternal bacterial translocation is a well-known phenomenon in animals outside of the mammalian class.[67]

Coincident with the notion of *in utero* gut colonization, has been the concept that with pregnancy, the composition of the maternal gut microbiome changes. It is well established that the mother makes physiological adaptations during pregnancy that are essential to meet the metabolic demands of pregnancy, and maternal intestinal adaptation is an area of budding research. Over the course of healthy human pregnancy two main adaptations have been demonstrated to occur in the maternal human gut; intestinal microbial load increases[68] and bacterial composition shifts.[58] In a landmark paper, Koren *et al.*[68] demonstrated that the pregnant microbiome shifts from first to third trimester of pregnancy, characterized by an increase in the abundance of *Proteobacteria* and *Actinobacteria*. Furthermore, colonization with first and third trimester human microbiota showed that third trimester microbiota induced insulin insensitivity and elevated adiposity in gnotobiotic mice compared with first trimester microbiota.[68] These unique observations suggest that the maternal gut microbiota may facilitate the necessary metabolic adaptations that occur late in pregnancy. Whether maternal gut barrier function and dendritic bacterial translocation similarly shift with normal advancing gestation to facilitate fetal gut colonization is currently unknown. Further, Koren *et al.* noted no significant changes in microbiota composition between the third trimester of pregnancy and 1 month postpartum, an observation that was repeated in one other study.[69] However, differences in microbial populations appear to differ between studies[68,69] and highlight the need for stringent and consistent molecular techniques and computational analyses when comparing data from different cohorts and studies. To date there are no animal data describing pregnancy-induced changes in the maternal gut microbiota over the course of pregnancy.

Postnatal gut colonization: mode of delivery and nutrition

Consistent with the Developmental Origins hypothesis that the prenatal and postnatal environments interact to establish adult phenotype, it may be that prenatal priming of the fetal gut modulates postnatal acquisition of an established microbiome. Unlike several mechanistic processes that have largely been studied in animal models, many studies describing the vertical transmission of maternal microbes at birth (vaginal microbiome *v.* C-section) and during lactation through breast milk, have been done in human cohorts.[15,70–72] Infants delivered vaginally harbor microbial communities similar to maternal vaginal populations, while microbes similar to the maternal epithelium dominates the intestinal tract of animals born by C-section.[73] *Lactobacillus*, *Prevotella* and *Sneathia* species dominate vaginally born infants while C-section infants harbor *Staphylococcus, Corynebacterium and Propionibacterium*.[73] This difference based on mode of delivery is speculated to be a result of the failure of contact between maternal vaginal and fecal flora.[74] Furthermore, differences between C-section and vaginally born infant microbial populations has been documented to persist 4[15] and 6 months after birth[75] and up to 7 years later.[76] Animal studies also show distinct differences in neonatal gut colonization as a result of mode of delivery. Within the first 2 weeks of life, vaginally delivered piglets shown higher proportions of *Bacteroides Prevotella* and *Clostridium* XIVa associated with higher levels of propionate and butyrate in the ascending colon.[77] Interestingly, gut colonization was independently modulated by postnatal nutrition, where sow-reared (breast fed) piglets showed higher levels of butyrate, isobutyrate, valerate and isovalerate concentrations *v.* formula-fed piglets,[77] suggesting that gut microbial metabolic activity differs between sow-reared and formula-fed piglets. Although not measured in this study, the presence of elevated proportions of *Bacteroides-Prevotella*, *Clostridium*, *Bifidobacterium* spp. and *Lactobacillus* spp. may explain the differing proportion of short-chain fatty acids (SCFAs). This result is consistent with human data that report distinct microbial profiles between breast and formula-fed infants.[72,78,79] Mice born by C-section, compared with spontaneous vaginal delivery, displayed a distinct bacterial profile at weaning characterized by a higher abundance of *Bacteroides* and *Lachnospiraceae* and reduced abundance of *Rikenellazeae* and *Ruminococcus*; however, no taxonomic clustering according to delivery method persisted to adulthood.[80] This appears to be in contrast to recent human data demonstrating that adults born by C-section have a distinctly different gut microbial composition.[81]

Postnatal gut colonization: exposure to prenatal antibiotics

Antibiotic treatment during pregnancy is commonly used to combat urinary and gastrointestinal infections,[82,83] bacterial vaginosis,[84] and to prevent maternal and infant infection during gestation and after delivery.[85] Antibiotic administration during pregnancy alters vaginal microbial populations,[86] contributes to aberrant microbial colonization of the infant gut[78,87,88] and has recently been associated with an increased risk of developing childhood obesity.[60] Given the close relationship between the gut microbiota and risk of obesity,[50] animal studies investigating broad-spectrum antibiotics during

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gestation have been used to understand the link between gut dysbiosis and long-term obesity risk (see next section). In rats, a single course of an antibiotic-cocktail of metronidazole, neomycin and polymyxin administered in drinking water 3 to 4 days before delivery resulted in higher stomach pH, lower stomach weight and increased intestinal permeability in neonates.[59] Treatment also promoted antibiotic-resistant *Enterobacteriaceae* in the offspring at birth, which persist beyond lactation.[59] Similar changes in long-term gut permeability have been demonstrated in pigs.[89] Increased gut permeability has been associated with increased bacterial translocation to metabolic tissues and in the promotion of non-alcoholic fatty liver disease (for review, see[90]), potentially linking aberrant neonatal gut colonization with long-term metabolic complications. Administration of similar antibiotic cocktails to non-obese diabetic (NOD) mice in early and late pregnancy induced a persistent reduction in offspring microbial diversity, distinct clustering of bacterial taxa and an increased risk of diabetes incidence observed at 20 weeks of age, but this disappeared at 30 weeks of age.[91] Collectively, these studies suggest that prenatal antibiotic administration induces permanent changes in both gut microbial populations and gut function, potentially contributing to an increased risk of developing metabolic complications in adulthood.

Early life modulation of gut colonization and risk of obesity and metabolic compromise

Maternal obesity and nutritional excess in large and small animal models

With our knowledge that different bacterial species utilize and produce distinct nutritional substrates and metabolites, and vary in their capacity to harvest energy,[10] the concept that obesity risk can be modulated by gut microbial communities has become plausible. An 'aberrant' gut microbiota is hypothesized to be equally as detrimental to organism health as having no microbiota at all.[59] Early genomic studies in male mice examining 16S ribosomal RNA gene sequences of cecal microbiota harvested from genetically obese ob/ob,[50] leptin deficient *db/db* mice, and models of diet-induced obesity[92,93] have collectively demonstrated shifts in the proportions of *Bacteroidetes* and *Firmicutes* relative to lean counterparts. These models showed a marked reduction and proportional increase in the levels of the *Bacteroidetes* and *Firmicutes* phyla, respectively.[50] Shotgun sequencing of the intestinal flora harvested from *ob/ob* mice display an enrichment of genes encoding enzymes involved in energy extraction, supporting the notion that the prominent bacterial populations of the obese gut specialize in energy harvest. Furthermore, upon colonization of germ-free mice with the distal gut microbiota harvested from conventionally raised mice, a dramatic increase in adiposity is observed within 10–14 days despite a decrease in food consumption {Turnbaugh, 2006 #11}. This change in adiposity is hypothesized to involve several related mechanisms including the microbial fermentation of indigestible dietary polysaccharides, subsequent intestinal absorption of monosaccharides and SCFAs, their conversion to more complex lipids in the liver and microbial regulation of host genes to promote the deposition of lipids into adipocytes {Turnbaugh, 2006 #11}. Collectively, these findings led researchers in the field to propose that the microbiota of obese individuals may be more efficient at extracting energy from a given diet than the microbiota of lean counterparts. Consistent results have been drawn from human work.[10]

Similar studies have yet to be conducted examining the evolution of the maternal obese microbiota throughout pregnancy in experimental animal models. In humans, two main factors have been identified that are associated with shifts in commensal populations in the maternal gut; pre-pregnancy weight and gestational weight gain. Examination of the microbiota of 36 normal weight [body mass index (BMI) < 25] and 18 overweight (BMI > 30) women demonstrated division-specific blooms of *Bacteroides* and *Staphylococcus* in overweight women, where proportions of *Bacteroides* was correlated to gestational weight gain.[58] Other studies have identified increases in *Staphylococcus*, *Enterobacteriaceae* and *Escherichia coli*, which have been reported to be strongly associated with metabolic endotoxaemia and inflammation. *Bifidobacterium* and *Akkermansia muciniphila*, responsible for the production of metabolites such as butyrate and mucin degradation, respectively, decrease in relative abundance in overweight pregnant women.[94] These results are in accordance with a recent study that displayed elevated levels of *Staphylococcus* and reduced levels of *Bifidobacterium* in the feces of infants, whom at the age of 7, were classified as clinically overweight.[94] Although elevated pre-pregnancy weight and gestational weight gain was associated with altered proportions of intestinal commensals, currently there are no data describing how the maternal microbiota evolves over the course of pregnancy in the context of obesity. Although existing data suggest a strong relationship between maternal obesity, gestational weight gain and gut microbial shifts, research has yet to reveal mechanisms explaining why these shifts occur and how microbial shifts are altered during obese pregnancy.

Despite our lack of understanding of the obese pregnant microbiome, studies describing gut microbial shifts and changes in gut function in offspring born to obese or diet-induced obese mothers have begun to emerge. Early studies have shown that maternal obesity induces ectopic adipose deposition in skeletal muscle[31] as well as enhanced expression of pro-inflammatory cytokines in fetal gut and young adult ovine offspring.[95] Indeed, free fatty acids can increase gut permeability to microbial products such as lipopolysaccharide (LPS), triggering gut inflammation[96] or can directly induce pattern recognition receptors (TLRs) and downstream proinflammatory pathways.[97] Despite the lack of microbial data, these early data collectively support the hypothesis that maternal obesity induces adaptive changes in fetal gut development that could predispose to gastrointestinal inflammatory disorders and to obesity.

In a murine model of maternal high fat diet, offspring born to mothers fed a high fat/high sugar diet throughout pregnancy had increased incidence of infection, autoimmune disorders and allergic sensitization associated with enhanced colonic inflammatory responses and increased circulating bacterial LPS.[98] These offspring also demonstrated decreased fecal bacterial diversity with an increased ratio of *Firmicutes* to *Bacteriodetes* and an over representation of the genera *Lachnospiraceae* and *Clostridiales*.[98] Although similar results were found in offspring of mice fed high omega-3 fatty acids during pregnancy, it appears that different fatty acids promote distinct shifts in gut microbiota as omega-3 pups demonstrated increases in *Blautia, Oscillibacter, Clostridales, Robinsoniella, Lactococcus* and *Eubacterium*, but a significant reduction in the abundance of *Lachnospiraceae, Anaerotruncus* and *Roseburia*,[99] highlighting the need to compare similar diets when comparing shifts in offspring microbial populations as a result of early life manipulations.

Despite increasing numbers of studies demonstrating that intrauterine exposure to an obesogenic environment not only predisposes offspring to systemic and organ-specific inflammation and gut dysbiosis, data on gut development and colonization derived from animal models must be interpreted with caution. Many model systems now exist contributing to our current understanding of host–microbiota interactions.[100] Rodents display unique gut microbial populations where the relative abundances of most of the dominant genera in mouse and human are quite different; genera with high abundance in human gut microbiota include *Prevotella*, *Faecalibacterium* and *Ruminococcus*, whereas *Lactobacillus*, *Alistipes* and *Turicibacter* are more abundant in mouse gut microbiota.[101] Rodents are also postnatal developers where rats and mice are born with immature gastrointestinal tracts that mature during a critical window within the first 10–14 days of postnatal life. However, despite these limitations, rodent models have contributed significantly to our understanding of the gut microbiome and its relationship to metabolic function and risk of obesity. Microbial manipulations essential to research are easily accomplished in small animals. Transgenic mice, gnotobiotic mice, as well as antibiotic administration and fecal colonization are easily performed in small animals that breed reasonably well in a short time period. Studies investigating ruminant species such as the sheep, which exhibit unique gut function[102] further contribute to our knowledge of species-specific shifts in microbial populations and gut susceptibilities. Thus, the advantages of using these types of animal models, as well as our detailed understanding of their biology, continue to make them indispensible in our investigations of early life programming of gut colonization and its relationship to long-term disease risk.

Although difficult to perform and costly, studies in non-human primates offer a close evolutionary relative and will significantly contribute to our understanding of early life impacts on human gut colonization and obesity risk. One such study has investigated the developmental programming of offspring gut colonization through maternal diet. As demonstrated by distinct clustering with principle coordinate analysis, maternal high-fat diet intake resulted in offspring gut dysbiosis, where the data that cluster tightly together and display the most significant dysbiosis was observed in offspring who experienced a high fat diet during fetal and postnatal life. Offspring demonstrated a depletion of *Proteobacteria*, specifically *Campylobacter* spp. and *Helicobacter* spp, and enrichment of the *Firmicutes, Ruminococcus* and *Dialister*.[103] It was proposed that the maternal diet, rather than maternal obesity *per se*, played a major role in shaping the offspring gut microbiome at 1 year of age.[103]

Although human studies are still lacking, a recent report has shown that maternal obesity is related to differences in the composition of the gut microbiota in children between 18 and 27 months of age even after accounting for paternal BMI and indicators of child body composition, suggesting that exposure rather than genetics may account for this shift in composition.[104] Interestingly, effects of maternal obesity on the composition of the gut microbiota in offspring were stronger and more consistent among those born to mothers of higher socioeconomic status (SES) as defined by income and/or education. Also, when PiCRUST was used to examine metagemone function based on obesity status in the higher income group only, no significant differences were found. Although the mechanisms underlying the interaction between maternal obesity and SES in predicting the composition of the child gut microbiota are not known, research investigating the relative contribution of genetic *v.* behavioral factors in obese adults may differ between individuals of high and low SES. Despite the fact that no differences in the children from obese and non-obese mothers were found in terms of breastfeeding behavior, age at which solid foods were introduced, or the current frequency of consumption of meat, vegetables and cereals, this study did not include detailed food diaries and it is possible that subtle changes in behavior and lifestyle could account for the differences in high *v.* low SES status in this population. The authors suggest that the extent to which maternal obesity confers measurable changes to the offspring gut microbiome may differ based on the etiology of maternal obesity. No doubt this area of research will continue to expand and provide us with a clearer understanding of the inter-relationship between lifestyle, behavior, maternal obesity, childhood obesity and their impacts on early life gut colonization.

Early life nutritional interventions: modifying gut colonization and obesity risk

Studies of host–microbe interactions early in life offer a new approach to investigate the mechanistic basis of obesity and related metabolic disorders. Although a number of nutritional interventions during the perinatal time window have emerged as beneficial[105–110], few have investigated impacts on early life gut colonization and function. Maternal dietary supplementation of methyl donors has been found to be effective in

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preventing early life-induced metabolic compromise and obesity, likely through changes in DNA methylation.[105,106,111,112] However, in rodents, contrary to the improvements seen in metabolic profiles, maternal methyl donor supplementation just before and throughout pregnancy and lactation (5 mg/kg folic acid; 0.5 g/kg vitamin B12; 5 g/kg betaine and 5.76 g/kg choline) significantly increased colitis susceptibility in offspring. This was associated with colonic mucosal DNA methylation.[113] In young adulthood, the offspring born to supplemented mothers show altered profiles of several microbial genera found in the colon.[113] *Roseburia*,[114] a genera which has previously been associated with Crohn's disease in humans, was also depleted. Furthermore, a maternal gluten-free diet during pregnancy and lactation in a NOD mouse model resulted in a shift in microbial populations of mothers and offspring. Offspring gut microbiota in *Verrcomicrobia* and *Proteobacteria* including *A. muciniphila* and *Proteus*.[115] Interestingly, these effects were seen only in offspring at weaning and not later in life, highlighting the role of nutrition in mediating gut colonization during the neonatal period. Long term, offspring showed a reduction in the incidence of diabetes, reduced pancreatic inflammation and increased gut tight junction and mucin expression levels, suggestive of enhanced gut barrier integrity.[115] Thus, *in utero* gluten-induced changes in early neonatal gut colonization appeared to confer improved gut function as well as long-term protective effect from diabetes in this model.

Despite our general lack of understanding of the specific signaling pathways involved, studies investigating perinatal probiotic supplementation have reinforced the notion that a balanced or 'healthy' microbiota early in life is critical in the maintenance of health (we use the term 'healthy' microbiota here to refer to those populations most similar to those that have been observed in lean, healthy individuals and animal models). Indeed, studies in human have shown that probiotic intake during pregnancy and lactation have positive outcomes in children including reduced incidence of atopy including eczema.[116,117] It appears, however, that strict administration during the prenatal period may not be enough and continued administration during lactation is required.[118] Apart from its role in nutrition, breast milk provides immune-modulatory components[119] and promotes neonatal gut colonization,[120] and thus could be a direct route for the delivery of prebiotics and probiotics to the infant in cases of ongoing maternal supplementation.[121] These data are supported by animals studies, where probiotic supplementation during pregnancy and lactation in pigs showed the presence of probiotic strains in the suckling piglets without direct administration,[122,123] although in animals probiotic exposure may also be through ingestion of the maternal feces.[124] In rats, probiotic supplementation to mothers late in pregnancy, throughout lactation and to their offspring demonstrated long-term effects on maternal and offspring microbial composition, weight gain and adiposity.[125] In contrast, a high protein or high-prebiotic-fibre diet throughout pregnancy and lactation in rats resulted in lower circulating LPS and higher *Bifidobacteria* in both mothers and offspring.[126] This result is not only attributed to the reported negative correlations between circulating LPS and *Bifidobacteria* levels but is explained by the introduction of critical growth substrates including human milk oligosaccharide (HMO), neutral oligosaccharide and reconstructed HMO metabolic pathways encoded by beneficial bacterial species.[127] It is known that specific endogenous bacteria such as *Bifidobacterium* species commonly found in the distal gastrointestinal tract of infants[127] such as *Bifidobacterium longum* subsp. *Infantis* utilize HMOs[128], thus it is possible that dietary combinations utilizling pre/probiotics that target these species, increase maternal transfer to breast milk and are beneficial in establishing early gut colonization. However, this has yet to be proven.

Interestingly, it has recently been shown that probiotic-induced changes in pup survival and sex ratio in mice was associated with endocrine-mediated changes in the mother. In mice lacking oxytocin, probiotic-induced microbial changes in the mother were absent.[129] Although the mechanisms are unknown, this observation is suggestive of an interaction between endocrine signaling and gut colonization during pregnancy.

Concluding remarks

The startling rise in the prevalence of obesity, particularly in women of reproductive age and children has made it a pressing global health concern. In order to combat this epidemic, current research targeting the early life environment has exploded in hopes of improving offspring metabolic outcome. Research on the maternal gut microbiota and fetal gut colonization is only in its infancy. The mechanisms driving maternal gut microbial shifts during healthy pregnancy and pregnancies complicated by obesity are currently unknown. Further investigation of the maternal gut microbiota throughout pregnancy is required to understand its influence on fetal gut establishment and development and whether disruption in the maternal microbiota due to obesity can confer increased risk of disease later in life in the offspring.

Given the recent association between bacterial colonization and metabolic/obesogenic outcomes, the current paradigm describing these diseases as 'non-communicable' may soon face formidable challenges. Although it has yet to be proven empirically whether the relationship between commensal bacterial transmission and obesity risk is direct or indirect, emerging evidence regarding modulation of maternal systems including the innate/adaptive immune response, nutrient uptake and utilization and/or inflammatory-mediated changes in gut function have demonstrated plausible mechanisms by which obesity may be considered 'communicable' between a mother and her offspring. The years of research to follow will doubtlessly shed much light on this new black box of microbial-induced phenotype modulation, and reveal new horizons for understanding the nature of metabolic disease transmission.

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Conflicts of Interest

None.

References

1. World Health Organization. *BMI Classification*. 2015. World Health Organization.
2. Hedley AA, Ogden CL, Johnson CL, *et al*. Prevalence of overweight and obesity among us children, adolescents, and adults, 1999-2002. *JAMA*. 2004; 291, 2847–2850.
3. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of childhood and adult obesity in the united states, 2011–2012. *JAMA*. 2014; 311, 806–814.
4. Catalano P, deMouzon SH. Maternal obesity and metabolic risk to the offspring: why lifestyle interventions may have not achieved the desired outcomes. *Int J Obes*. 2015; 39, 642–649.
5. Athukorala C, Rumbold A, Willson K, Crowther C. The risk of adverse pregnancy outcomes in women who are overweight or obese. *BMC Pregnancy Childbirth*. 2010; 10, 56.
6. Crane JM, Murphy P, Burrage L, Hutchens D. Maternal and perinatal outcomes of extreme obesity in pregnancy. *J Obstet Gynaecol Can*. 2013; 35, 606–611.
7. Portela DS, Vieira TO, Matos SM, de Oliveira NF, Vieira GO. Maternal obesity, environmental factors, cesarean delivery and breastfeeding as determinants of overweight and obesity in children: results from a cohort. *BMC Pregnancy Childbirth*. 2015; 15, 94.
8. Gluckman P, Hanson M. *The Developmental Origins of Health and Disease*. 2006. Springer: New York.
9. Gluckman PD, Hanson MA. The developmental origins of the metabolic syndrome. *Trends Endocrinol Metab*. 2004; 15, 183–187.
10. Turnbaugh PJ, Ley RE, Mahowald MA, *et al*. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006; 444, 1027–1031.
11. Henao-Mejia J, Elinav E, Jin C, *et al*. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature*. 2012; 482, 179–185.
12. Aagaard K, Ma J, Antony KM, *et al*. The placenta harbors a unique microbiome. *Sci Trans Med* 2014; 6, 237ra65.
13. Madan JC, Salari RC, Saxena D, *et al*. Gut microbial colonisation in premature neonates predicts neonatal sepsis. *Arch Dis Child Fetal Neonatal Ed*. 2012; 97, F456–F462.
14. Gritz EC, Bhandari V. The human neonatal gut microbiome: a brief review. *Front Pediatr*. 2015; 3, 17.
15. Azad MB, Konya T, Maughan H, *et al*. Gut microbiota of healthy Canadian infants: profiles by mode of delivery and infant diet at 4 months. *Can Med Assoc J*. 2013; 185, 385–394.
16. Barker DJ. Childhood causes of adult diseases. *Arch Dis Child*. 1988; 63, 867–869.
17. Barker DJ, Winter PD, Osmond C, Margetts B, Simmonds SJ. Weight in infancy and death from ischaemic heart disease. *Lancet*. 1989; 2, 577–580.
18. Barker DJP. Fetal origins of coronary heart disease. *BMJ*. 1995; 311, 171–174.
19. Howie GJ, Sloboda DM, Kamal T, Vickers MH. Maternal nutritional history predicts obesity in adult offspring independent of postnatal diet. *J Physiol*. 2009; 587, 905–915.
20. Zheng J, Xiao X, Zhang Q, *et al*. Maternal high-fat diet modulates hepatic glucose, lipid homeostasis and gene expression in the PPAR pathway in the early life of offspring. *Int J Mol Sci*. 2014; 15, 14967–14983.
21. Shankar K, Harrell A, Liu X, *et al*. Maternal obesity at conception programs obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol*. 2008; 294, R528–R538.
22. Page KC, Malik RE, Ripple JA, Anday EK. *Maternal and postweaning diet interaction alters hypothalamic gene expression and modulates response to a high-fat diet in male offspring*. 2009; 297, R1049–R1057.
23. Samuelsson A-M, Matthews PA, Argenton M, *et al*. Diet-induced obesity in female mice leads to offspring hyperphagia, adiposity, hypertension, and insulin resistance: a novel murine model of developmental programming. *Hypertension*. 2008; 51, 383–392.
24. Nivoit P, Morens C, Van Assche FA, *et al*. Established diet-induced obesity in female rats leads to offspring hyperphagia, adiposity and insulin resistance. *Diabetologia*. 2009; 52, 1133–1142.
25. Howie GJ, Sloboda DM, Reynolds CM, Vickers MH. Timing of maternal exposure to a high fat diet and development of obesity and hyperinsulinemia in male rat offspring: same metabolic phenotype, different developmental pathways? *J Nutr Metab*. 2013; 2013, 517384.
26. Taylor PD, McConnell J, Khan IY, *et al*. Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy. *Am J Physiol Integr Comp Physiol*. 2005; 288, R134–R139.
27. Borengasser SJ, Lau F, Kang P, *et al*. Maternal obesity during gestation impairs fatty acid oxidation and mitochondrial SIRT3 expression in rat offspring at weaning. *PLoS One*. 2011; 6, e24068.
28. Oben JA, Mouralidarane A, Samuelsson A-M, *et al*. Maternal obesity during pregnancy and lactation programs the development of offspring non-alcoholic fatty liver disease in mice. *J Hepatol*. 2010; 52, 913–920.
29. Bruce KD, Cagampang FR, Argenton M, *et al*. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. *Hepatology*. 2009; 50, 1796–1808.
30. Connor KL, Vickers MH, Beltrand J, Meaney MJ, Sloboda DM. Nature, nurture or nutrition? Impact of maternal nutrition on maternal care, offspring development and reproductive function. *J Physiol*. 2012; 590, 2167–2180.
31. Yan X, Huang Y, Zhao J-X, *et al*. Maternal obesity-impaired insulin signaling in sheep and induced lipid accumulation and fibrosis in skeletal muscle of offspring. *Biol Reprod*. 2011; 85, 172–178.
32. Zhu MJ, Han B, Tong J, *et al*. AMP-activated protein kinase signalling pathways are down regulated and skeletal muscle development impaired in fetuses of obese, over-nourished sheep. *J Physiol*. 2008; 586, 2651–2664.
33. Yan X, Zhu MJ, Xu W, *et al*. Up-regulation of toll-like receptor 4/nuclear factor-κB signaling is associated with enhanced adipogenesis and insulin resistance in fetal skeletal muscle of obese sheep at late gestation. *Endocrinology*. 2010; 151, 380–387.
34. McCurdy CE, Bishop JM, Williams SM, *et al*. Maternal high-fat diet triggers lipotoxicity in the fetal livers of nonhuman primates. *J Clin Invest*. 2009; 119, 323–335.

35. Conner B, Hetrik B, Chicco A, McCurdy C. Maternal obesity alters offspring muscle mitochondrial function in nonhuman primates. *FASEB J*. 2015; 29, 1036–1037.
36. Fan L, Lindsley SR, Comstock SM, *et al*. Maternal high-fat diet impacts endothelial function in nonhuman primate offspring. *Int J Obes*. 2013; 37, 254–262.
37. Grant WF, Gillingham MB, Batra AK, *et al*. Maternal high fat diet is associated with decreased plasma n-3 fatty acids and fetal hepatic apoptosis in nonhuman primates. *PLoS One*. 2011; 6, e17261.
38. Grant WF, Nicol LE, Thorn SR, *et al*.. Perinatal exposure to a high-fat diet is associated with reduced hepatic sympathetic innervation in one-year old male Japanese macaques. *PLoS One*. 2012; 7, e48119.
39. Suter MA, Chen A, Burdine MS, *et al*. A maternal high-fat diet modulates fetal SIRT1 histone and protein deacetylase activity in nonhuman primates. *FASEB J*. 2012; 26, 5106–5114.
40. Whitman WB, Coleman DC, Wiebe WJ. Prokaryotes: the unseen majority. *Proc Natl Acad Sci*. 1998; 95, 6578–6583.
41. Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK. Chapter 8 – Host–bacterial symbiosis in health and disease. In *Advances in Immunology* (eds. Sidonia F, Andrea C), 2010; pp. 243–274. Academic Press: Pasadena, CA.
42. Backhed F, Ding H, Wang T, *et al*. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004; 101, 15718–15723.
43. Nieuwdorp M, Gilijamse PW, Pai N, Kaplan LM. Role of the microbiome in energy regulation and metabolism. *Gastroenterology*. 2014; 146, 1525–1533.
44. Hooper LV, Macpherson AJ. Immune adaptations that maintain homeostasis with the intestinal microbiota. *Nat Rev Immunol*. 2010; 10, 159–169.
45. Turner JR. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol*. 2009; 9, 799–809.
46. Reyniers JA. The pure-culture concept and gnotobiotics. *Ann N Y Acad Sci*. 1959; 78, 3–16.
47. Thompson GR, Trexler PC. Gastrointestinal structure and function in germ-free or gnotobiotic animals. *Gut*. 1971; 12, 230–235.
48. Duncan SH, Lobley GE, Holtrop G, *et al*. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes*. 2008; 32, 1720–1724.
49. Ley RE. Obesity and the human microbiome. *Curr Opin Gastroenterol*. 2010; 26, 5–11.
50. Ley RE, Backhed F, Turnbaugh P, *et al*. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A*. 2005; 102, 11070–11075.
51. Tissier H. *Recherches Sur la blore Intestinales des Nourrisons (etat Normal et Pathologiques)*. 1900. G. Carré et C. Naud: Paris, France.
52. Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. *BJOG*. 2003; 110, 71–75.
53. Schaedler RW, Dubos R, Costello R. The development of the bacterial flora in the gastrointestinal tract of mice. *J Exp Med*. 1965; 122, 59–66.
54. Methe B. A framework for human microbiome research. *Nature*. 2012; 486, 215–221.
55. Jiménez E, Marín ML, Martín R, *et al*. Is meconium from healthy newborns actually sterile? *Res Microbiol*. 2008; 159, 187–193.
56. Moles L, Gómez M, Heilig H, *et al*. Bacterial diversity in meconium of preterm neonates and evolution of their fecal microbiota during the first month of life. *PLoS One*. 2013; 8, e66986.
57. Jiménez E, Fernández L, Marín M, *et al*. Isolation of commensal bacteria from umbilical cord blood of healthy neonates born by cesarean section. *Curr Microbiol*. 2005; 51, 270–274.
58. Collado MC, Isolauri E, Laitinen K, Salminen S. Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. *Am J Clin Nutr*. 2008; 88, 894–899.
59. Fak F, Ahrne S, Molin G, Jeppsson B, Westrom B. Microbial manipulation of the rat dam changes bacterial colonization and alters properties of the gut in her offspring. *Am J Physiol Gastrointest Liver Physiol*. 2008; 294, G148–G154.
60. Mueller NT, Whyatt R, Hoepner L, *et al*. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int J Obes*. 2015; 39, 665–670.
61. Pacha J. Development of intestinal transport function in mammals. *Physiol Rev*. 2000; 80, 1633–1667.
62. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr*. 1999; 69, 1035s–1045ss.
63. Perez PF, Doré J, Leclerc M, *et al*. Bacterial imprinting of the neonatal immune system: lessons from maternal cells? *Pediatrics*. 2007; 119, e724–e732.
64. Rescigno M, Rotta G, Valzasina B, Ricciardi-Castagnoli P. Dendritic cells shuttle microbes across gut epithelial monolayers. *Immunobiology*. 2001; 204, 572–581.
65. Rescigno M, Urbano M, Valzasina B, *et al*. Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol*. 2001; 2, 361–367.
66. Jin L, Hinde K, Tao L. Species diversity and relative abundance of lactic acid bacteria in the milk of rhesus monkeys (*Macaca mulatta*). *J Med Primatol*. 2011; 40, 52–58.
67. Funkhouser LJ, Bordenstein SR. Mom knows best: the universality of maternal microbial transmission. *PLoS Biol*. 2013; 11, e1001631.
68. Koren O, Goodrich JK, Cullender TC, *et al*. Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell*. 2012; 150, 470–480.
69. Jost T, Lacroix C, Braegger C, Chassard C. Stability of the maternal gut microbiota during late pregnancy and early lactation. *Curr Microbiol*. 2014; 68, 419–427.
70. Makino H, Kushiro A, Ishikawa E, *et al*. Mother-to-infant transmission of intestinal bifidobacterial strains has an impact on the early development of vaginally delivered infant's microbiota. *PLoS One*. 2013; 8, e78331.
71. Biasucci G, Rubini M, Riboni S, *et al*. Mode of delivery affects the bacterial community in the newborn gut. *Early Hum Dev*. 2010; 86(Suppl 1), 13–15.
72. Bezirtzoglou E, Tsiotsias A, Welling GW. Microbiota profile in feces of breast- and formula-fed newborns by using fluorescence in situ hybridization (FISH). *Anaerobe*. 2011; 17, 478–482.
73. Dominguez-Bello MG, Costello EK, Contreras M, *et al*. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A*. 2010; 107, 11971–11975.
74. Murono K, Fujita K, Yoshikawa M, *et al*. Acquisition of nonmaternal enterobacteriaceae by infants delivered in hospitals. *J Pediatr*. 1993; 122, 120–125.

75. Gronlund MM, Lehtonen OP, Eerola E, Kero P. Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr*. 1999; 28, 19–25.
76. Salminen S, Gibson GR, McCartney AL, Isolauri E. Influence of mode of delivery on gut microbiota composition in seven year old children. *Gut*. 2004; 53, 1388–1389.
77. Wang M, Radlowski EC, Monaco MH, *et al*. Mode of delivery and early nutrition modulate microbial colonization and fermentation products in neonatal piglets. *J Nutr*. 2013; 143, 795–803.
78. Fallani M, Young D, Scott J, *et al*. Intestinal microbiota of 6-week-old infants across Europe: geographic influence beyond delivery mode, breast-feeding, and antibiotics. *J Pediatr Gastroenterol Nutr*. 2010; 51, 77–84.
79. Gomez-Llorente C, Plaza-Diaz J, Aguilera M, *et al*. Three main factors define changes in fecal microbiota associated with feeding modality in infants. *J Pediatr Gastroenterol Nutr*. 2013; 57, 461–466.
80. Hansen CH, Andersen LS, Krych L, *et al*. Mode of delivery shapes gut colonization pattern and modulates regulatory immunity in mice. *J Immunol*. 2014; 193, 1213–1222.
81. Goedert JJ, Hua X, Yu G, Shi J. Diversity and composition of the adult fecal microbiome associated with history of cesarean birth or appendectomy: analysis of the American gut project. *EBioMedicine*. 2014; 1, 167–172.
82. Le J, Briggs GG, McKeown A, Bustillo G. Urinary tract infections during pregnancy. *Ann Pharmacother*. 2004; 38, 1692–1701.
83. Chan BT, Hohmann E, Barshak MB, Pukkila-Worley R. Treatment of listeriosis in first trimester of pregnancy. *Emerg Infect Dis*. 2013; 19, 839–841.
84. Sheehy O, Santos F, Ferreira E, Berard A. The use of metronidazole during pregnancy: a review of evidence. *Curr Drug Saf*. 2015; 10, 170–179.
85. Turrentine MA, Greisinger AJ, Brown KS, Wehmanen OA, Mouzoon ME. Duration of intrapartum antibiotics for group B streptococcus on the diagnosis of clinical neonatal sepsis. *Infect Dis Obstet Gynecol*. 2013; 2013, 525878.
86. Stokholm J, Schjorring S, Eskildsen CE, *et al*. Antibiotic use during pregnancy alters the commensal vaginal microbiota. *Clin Microbiol Infect*. 2014; 20, 629–635.
87. Jauréguy F, Carton M, Panel P, *et al*. Effects of intrapartum penicillin prophylaxis on intestinal bacterial colonization in infants. *J Clin Microbiol*. 2004; 42, 5184–5188.
88. Mangin I, Suau A, Gotteland M, Brunser O, Pochart P. Amoxicillin treatment modifies the composition of Bifidobacterium species in infant intestinal microbiota. *Anaerobe*. 2010; 16, 433–438.
89. Arnal M-E, Zhang J, Erridge C, Smidt H, Lallès J-P. Maternal antibiotic-induced early changes in microbial colonization selectively modulate colonic permeability and inducible heat shock proteins, and digesta concentrations of alkaline phosphatase and TLR-Stimulants in swine offspring. *PLoS One*. 2015; 10, e0118092.
90. Scarpellini E, Lupo M, Iegri C, *et al*. Intestinal permeability in non-alcoholic fatty liver disease: the gut-liver axis. *Rev Recent Clin Trials*. 2014; 9, 141–147.
91. Tormo-Badia N, Hakansson A, Vasudevan K, *et al*. Antibiotic treatment of pregnant non-obese diabetic mice leads to altered gut microbiota and intestinal immunological changes in the offspring. *Scand J Immunol*. 2014; 80, 250–260.
92. Turnbaugh PJ, Ridaura VK, Faith JJ, *et al*. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. *Sci Transl Med* 2009; 1, 6ra14.
93. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe*. 2008; 3, 213–223.
94. Santacruz A, Collado MC, Garcia-Valdes L, *et al*. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr*. 2010; 104, 83–92.
95. Yan X, Huang Y, Wang H, *et al*. Maternal obesity induces sustained inflammation in both fetal and offspring large intestine of sheep. *Inflamm Bowel Dis*. 2011; 17, 1513–1522.
96. Stenman LK, Holma R, Korpela R. High-fat-induced intestinal permeability dysfunction associated with altered fecal bile acids. *World J Gastroenterol*. 2012; 18, 923–929.
97. Huang S, Rutkowsky JM, Snodgrass RG, *et al*. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. *J Lipid Res*. 2012; 53, 2002–2013.
98. Myles IA, Fontecilla NM, Janelsins BM, *et al*. Parental dietary fat intake alters offspring microbiome and immunity. *J Immunol (Baltimore, Md: 1950)*. 2013; 191. 10.4049/jimmunol.1301057.
99. Myles IA, Pincus NB, Fontecilla NM, Datta SK. Effects of parental omega-3 fatty acid intake on offspring microbiome and immunity. *PLoS One*. 2014; 9, e87181.
100. Kostic AD, Howitt MR, Garrett WS. Exploring host–microbiota interactions in animal models and humans. *Genes Dev*. 2013; 27, 701–718.
101. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech*. 2015; 8, 1–16.
102. Hofmann RR. Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. *Oecologia*. 1989; 78, 443–457.
103. Ma J, Prince AL, Bader D, *et al*. High-fat maternal diet during pregnancy persistently alters the offspring microbiome in a primate model. *Nat Commun*. 2014; 5, 3889.
104. Galley JD, Bailey M, Kamp Dush C, Schoppe-Sullivan S, Christian LM. Maternal obesity is associated with alterations in the gut microbiome in toddlers. *PLoS One*. 2014; 9, e113026.
105. Lillycrop KA, Phillips ES, Jackson AA, Hanson MA, Burdge GC. Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring. *J Nutr*. 2005; 135, 1382–1386.
106. Waterland RA, Travisano M, Tahiliani KG, Rached MT, Mirza S. Methyl donor supplementation prevents transgenerational amplification of obesity. *Int J Obes*. 2008; 32, 1373–1379.
107. Stoffers DA, Desai BM, DeLeon DD, Simmons RA. Neonatal exendin-4 prevents the development of diabetes in the intrauterine growth retarded rat. *Diabetes*. 2003; 52, 734–740.
108. Fisher RE, Or'Rashid M, Quinton M, *et al*. Maternal supplementation with fishmeal protects against late gestation endotoxin-induced fetal programming of the ovine hypothalamic-pituitary-adrenal axis. *J Dev Orig Health Dis*. 2014; 5, 206–213.
109. Gray C, Vickers MH, Segovia SA, Zhang XD, Reynolds CM. A maternal high fat diet programmes endothelial function and cardiovascular status in adult male offspring independent of

body weight, which is reversed by maternal Conjugated Linoleic Acid (CLA) supplementation. *PLoS One*. 2015; 10, e0115994.
110. Benaissa N, Merzouk H, Merzouk SA, Narce M. Effects of maternal linseed oil supplementation on metabolic parameters in cafeteria diet-induced obese rats. *Biomed Environ Sci*. 2015; 28, 298–302.
111. Carlin J, George R, Reyes TM. Methyl donor supplementation blocks the adverse effects of maternal high fat diet on offspring physiology. *PLoS One*. 2013; 8, e63549.
112. Cordero P, Milagro FI, Campion J, Martinez JA. Supplementation with methyl donors during lactation to high-fat-sucrose-fed dams protects offspring against liver fat accumulation when consuming an obesogenic diet. *J Dev Orig Health Dis*. 2014; 5, 385–395.
113. Schaible TD, Harris RA, Dowd SE, Smith CW, Kellermayer R. Maternal methyl-donor supplementation induces prolonged murine offspring colitis susceptibility in association with mucosal epigenetic and microbiomic changes. *Hum Mol Genet*. 2011; 20, 1687–1696.
114. Willing BP, Dicksved J, Halfvarson J, *et al*. A Pyrosequencing study in twins shows that gastrointestinal microbial profiles vary with inflammatory bowel disease phenotypes. *Gastroenterology*. 2010; 139, 1844–54.e1.
115. Hansen CHF, Krych Ł, Buschard K, *et al*. A maternal gluten-free diet reduces inflammation and diabetes incidence in the offspring of NOD mice. *Diabetes*. 2014; 63, 2821–2832.
116. Rautava S, Kainonen E, Salminen S, Isolauri E. Maternal probiotic supplementation during pregnancy and breast-feeding reduces the risk of eczema in the infant. *J Allergy Clin Immunol*. 2012; 130, 1355–1360.
117. Rautava S, Kalliomäki M, Isolauri E. Probiotics during pregnancy and breast-feeding might confer immunomodulatory protection against atopic disease in the infant. *J Allergy Clin Immunol*. 2002; 109, 119–121.
118. Boyle RJ, Ismail IH, Kivivuori S, *et al*. Lactobacillus GG treatment during pregnancy for the prevention of eczema: a randomized controlled trial. *Allergy*. 2011; 66, 509–516.
119. Field CJ. The immunological components of human milk and their effect on immune development in infants. *J Nutr*. 2005; 135, 1–4.
120. Gueimonde M, Laitinen K, Salminen S, Isolauri E. Breast milk: a source of bifidobacteria for infant gut development and maturation? *Neonatology*. 2007; 92, 64–66.
121. Abrahamsson TR, Sinkiewicz G, Jakobsson T, Fredrikson M, Björkstén B. Probiotic lactobacilli in breast milk and infant stool in relation to oral intake during the first year of life. *J Pediatr Gastroenterol Nutr*. 2009; 49, 349–354.
122. Starke IC, Pieper R, Neumann K, Zentek J, Vahjen W. Individual responses of mother sows to a probiotic Enterococcus faecium strain lead to different microbiota composition in their offspring. *Benef Microbes*. 2013; 4, 345–356.
123. Taras D, Vahjen W, Macha M, Simon O. Response of performance characteristics and fecal consistency to long-lasting dietary supplementation with the probiotic strain *Bacillus cereus* var. toyoi to sows and piglets. *Arch Anim Nutr*. 2005; 59, 405–417.
124. Jadamus A, Vahjen W, SchÄFer K, Simon O. Influence of the probiotic strain *Bacillus cereus* var. toyoi on the development of enterobacterial growth and on selected parameters of bacterial metabolism in digesta samples of piglets. *J Anim Physiol Anim Nutr (Berl)*. 2002; 86, 42–54.
125. Karlsson CL, Molin G, Fak F, *et al*. Effects on weight gain and gut microbiota in rats given bacterial supplements and a high-energy-dense diet from fetal life through to 6 months of age. *Br J Nutr*. 2011; 106, 887–895.
126. Hallam MC, Barile D, Meyrand M, German JB, Reimer RA. Maternal high-protein or high-prebiotic-fiber diets affect maternal milk composition and gut microbiota in rat dams and their offspring. *Obesity*. 2014; 22, 2344–2351.
127. Sela DA, Li Y, Lerno L, *et al*. An infant-associated bacterial commensal utilizes breast milk sialyloligosaccharides. *J Biol Chem*. 2011; 286, 11909–11918.
128. Oozeer R, van Limpt K, Ludwig T, *et al*. Intestinal microbiology in early life: specific prebiotics can have similar functionalities as human-milk oligosaccharides. *Am J Clin Nutr*. 2013; 98, 561S–571SS.
129. Ibrahim Y, Kearney SM, Levkovich T, *et al*. Maternal gut microbes control offspring sex and survival. *J Probiotics Health*. 2015; 2, 120.
130. Abecia L, Fondevila M, Balcells J, McEwan NR. The effect of lactating rabbit does on the development of the caecal microbial community in the pups they nurture. *J Appl Microbiol*. 2007; 103, 557–564.