Journal of Developmental Origins of Health and Disease (2016), **7**(1), 2–4.

doi:10.1017/S2040174415007916

PREFACE

Perinatal programming of gut microbiota and immunity

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The developing organism uses environmental information, which may include nutrient and hormone levels during critical windows of preconceptional, prenatal and early postnatal life, to change its developmental trajectory to ensure survival. Although initially focused on early life nutrition as a pathway to obesity and related metabolic abnormalities, the Developmental Origins of Health and Disease (DOHaD) approach to understanding disease risk has expanded to include maternal health conditions during pregnancy, and the psychobiological impacts of maternal and infant stress. With the advent of new gene sequencing technology, we are better able to characterize microbial communities in various human biological samples to elucidate pathways between pre and postnatal environmental stimuli, microbial community composition and disease outcomes. In this regard, host–bacterial relationships have now been identified as modulators of disease risk, and links between maternal gut, breast milk and infant gut microbial composition have been identified as novel agents for developmental programming. Although this field of research is only just beginning to uncover new and exciting mechanisms and pathways, it is exciting to consider role of early life changes in the mother's and the infant's gut microbiota as another agent mediating disease risk.

In this regard, early life gut colonization could influence metabolic tissue development in determining chronic disease risk. Although the maternal–placental–fetal unit ultimately dictates the growth and development of the fetus, we can no longer ignore the large number of bacteria that inhabit the maternal–child environment and likely play a key role in our predisposition to disease risk. These microbiota are responsive to their environment where changes in the developing gut niche, be it inflammatory-, endocrine- or nutritionally mediated, may ultimately impact on maternal adaptation to pregnancy, the function of the placenta, the growth of the fetus, breast milk composition and gut colonization early in life. It is these nuanced changes in our commensal bacteria that may indeed play some role in imparting an increased risk of obesity and metabolic function in our life-long risk of disease. In fact, gut microbes during infancy have been identified as necessary for the maturation of the gut immune and metabolic systems. Both gut microbiota profiles and exposures that cause microbiota dysbiosis, such as infant antibiotic use, have been implicated with the development of allergy, asthma and overweight.

Without doubt, infant gut microbial diversity and richness responds rapidly to changes in the postnatal diet and environment. By the end of the 1st year of life, gut microbial composition closely resembles that of an adult. Yet, early microbial colonization also plays a key role in this transition. Several new publications from the GUSTO and ALADDIN cohorts have identified, for example, that greater initial abundance of *Streptococcus* in newborn microbiota can delay bifidobacterial colonization and that formula feeding soon after birth has a profound influence on microbial composition 6 months later. Recent findings suggest that fetal microbial exposure during pregnancy may be even more important for programming infant gut microbial composition. Bacterial deoxyribonucleic acid (DNA) has now been detected in the placenta, amniotic fluid, umbilical cord and meconium (first stool after birth) in newborn infants. New data also show that maternal gut bacterial load increases over the course of pregnancy and bacterial populations shift with advancing gestation. How this remodeling of maternal gut microbiome over the course of pregnancy influences placental and/or fetal development has yet to be elucidated. It is clear, however, that these microbial–host relationships impose an additional, previously unidentified, factor in the programming of postnatal health and disease risk. For interventions which alter gut microbiota, such as probiotic supplementation, administration during pregnancy is required for effectiveness in allergy prevention. Further, we are now learning that commensal microbes are present in breast milk and composition differs according to maternal health conditions. Breast milk composition, too, can be altered as evident by the presence of the probiotic, *Lactobacillus*, in colostrum (first breast milk after birth) after supplementation to pregnant mothers.

Adopting a DOHaD perspective, we have compiled a collection of five 'thinking out of the box' papers to illustrate fetal programming of mother's breast milk, and the gut microbiome and immunity in the infant. Evidence will be presented on the microbiota and immune composition of meconium, fecal samples in infants by 4 months of age, and mother's colostrum and breast milk 1 month after birth in relation to pregnancy conditions (maternal parity, overweight, vaginal group B *Streptococcus* (GBS) colonization and sex of the fetus), and birth events (cesarean section). Highlights of these papers and how they complement each other are presented below.

Part of this collection also includes state-of-the-art reviews on these themes from the human and animal experimental literature. The story begins with the overview provided by Rautava.

Largely forgotten in the world of early origins until recently, Rautava underscores that intimate contact with the microbial world is necessary for normal development in early life. Without this contact we see defective maturation of the gastrointestinal tract and immune systems particularly associated with allergic-type adaptive immune responses. Highlighted in the review are factors known to perturb early gut colonization on later health: mode of delivery and early antibiotic exposure are associated with deviations in what is considered to be 'normal' colonization in vaginal deliveries not complicated by antibiotic use; deviations that are associated with compromised immunity in childhood. The role of breast milk as a modulator of the neonatal gut microbiome is a focus of Rautava's review; breast milk contains important fuels that encourage the growth of beneficial bacteria in neonates and may even provide some key commensals, although thus far the evidence is circumstantial. The composition of the breast milk microbiome is influenced by a number of factors, including maternal metabolic status, length of gestation and of breastfeeding, mode of delivery and is purported to have important immunomodulatory properties, thought to be mediated via transforming growth factor (TGF)-β. As gut microbial composition is associated with immunomodulation of child health, it may not be surprising that interventions in the pregnant and breastfeeding mother have targeted the microbiome through administration of probiotics. Although it appears that probiotics appear safe and effective in reducing some atopic conditions in children, there is a paucity of data on whether this intervention has long-term benefits in children.

The concept of an early life origin of obesity has been well established and much of what is understood regarding the association between metabolic function and dysfunction is based upon landmark studies in animal models. Sloboda *et al.* present an overview of the contribution of experimental modulation of the gut microbiome to our understanding of the programming of metabolic disease in offspring. Studies in adult male gnotobiotic mice (mice devoid of all bacteria) have served as a platform for understanding the role of commensal bacteria in modulating the obesogenic phenotype. With no commensal bacteria, these mice remain lean on a high-fat diet, indeed colonization of germ-free mice with gut microbiota harvested from conventionally raised mice results in increased adiposity. With this knowledge the concept that obesity risk can be modulated by gut microbial communities became plausible, where an 'aberrant' gut microbiota appears to be as detrimental to metabolic health as having no microbiota at all. Early genomic studies and models of diet-induced obesity have collectively demonstrated shifts in the proportions of key commensals, and similar observations have been shown in obese pregnancies. Not surprising then is the explosion of animal models describing the developmental programming of offspring gut colonization through pregnancy-specific manipulations, including antibiotic use, maternal diet and/or obesity. Although as of yet immature, this area of research has begun to suggest that these types of manipulations may impose increased risk of disease through the modulation of postnatal gut colonization. This concept thus opens the door to novel interventions targeted at modulating gut bacterial composition, including probiotics.

Kozyrskyj *et al.* take a human perspective in their review, focusing on the role of the gut microbiome in mediating this early life predisposition to obesity. The concept that increased risk of disease through the modulation of postnatal gut colonization is highlighted in studies in children describing shifts in the levels of bacterial metabolites/short chain fatty acids in obese children compared with normal weight children, and in those describing gut dysbiosis in children before the onset of an outward obese phenotype. Despite these thought-provoking studies, however, it remains to be determined whether these shifts in microbial populations are the cause or just a response to changing metabolic conditions. Modulating factors that impose constraints on the microbial association in overweight and obesity no doubt contribute to the complexity of this issue; sexual dimorphism has been shown in studies of antibiotic use and breastfeeding in infants as well as ethnicity and geographical location. Despite these limitations, the authors postulate that accumulating data point toward the colonization of the neonatal gut with specific bacterial species to predict risk for child overweight, and that this relationship is modulated by pregnancy overweight, and may be related to postnatal events, including cesarean section delivery, maternal and infant antibiotic treatment, and breastfeeding.

Finally, the concept that the mother provides a seeding population of microbes to the fetus is an emerging theory that rebuts the previous notion that the fetus develops in a sterile environment. All three review papers highlight studies that have shown the presence of bacterial DNA in amniotic fluid, umbilical cord, the placenta, as well as meconium – bacteria that are closely related to commensals found normally in the gut. Indeed, the concept that the 'pregnant microbiome' contributes to not only maternal adaptation to pregnancy but also may mediate the intrauterine environment within which the fetus develops is the subject of intense research. Although it has yet to be proven whether the relationship between early life 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. Thus, studies of host–microbe interactions early in life offer a new approach to investigate the mechanistic basis of obesity and related metabolic disorders.

In this special issue, we have solicited five papers to further our understanding on the role of perinatal programming of gut microbiota and the host–microbe interface during infancy. Some of these authors have been some of the first

https://doi.org/10.1017/S2040174415007916 Published online by Cambridge University Press

to contribute evidence on newborn meconium and breast milk microbiota.

Antibiotic resistance genes are present in newborn meconium: evidence of maternal transmission?

Gut microbiota has been identified as an important reservoir of antibiotic resistance genes (ARGs). Gosalbes *et al.* screened meconium samples from the INMA (Infancia y Medio Ambiente – Childhood and the Environment) cohort, as well as fecal samples of 1-week-old infants, maternal stool and colostrum (first milk) from a smaller birth cohort in the same Spanish region, for genes conferring resistance to β-lactam antibiotics (BLr) and tetracycline (Tcr). BLr and Tcr were highly prevalent in meconium and early fecal samples. ARGs in mother's stool before delivery reached newborn meconium and mother's colostrum, implying that the gut resistance reservoir starts to accumulate before birth. Levels of the mecA gene, which confers BLr to methicillin-resistant *Staphylococcus aureus*, responsible for difficult-to-treat infections, were six-fold higher in meconium and 1-week-old samples than detected in mothers. This resistance gene was detected almost exclusively in meconium samples colonized with genus *Streptococcus* and in all of meconium following cesarean delivery.

Maternal vaginal GBS colonization affects microbial composition of the early infant gut

Although GBS (group B *Streptococcus* or *Streptococcus agalactiae*) vaginal colonization during pregnancy is becoming more prevalent, it is unknown whether GBS presence in vaginal microbiota influences the infant gut microbiome. Cassidy-Bushrow *et al.* analyzed stool specimens from 262 infants at 1 or 6 months of age in the general population WHEALS (Wayne County Health, Environment, Allergy and Asthma Longitudinal Study) cohort, which were sequenced at V4 by Illumina MiSeq. Similar to other populations, about one-third of women in this U.S. study were GBS positive, of whom 72.5% received *intrapartum* antibiotics. Infants born to GBS-positive mothers were more likely to have GBS detected in their stool 1 month after vaginal birth but not after cesarean delivery, implicating transfer during the birth process. They also tended to have a higher abundance of Streptococcaceae at 6 months of age, a profile which has been observed to predict adiposity later in infancy.

Cesarean section delivery affects microbial composition in early breast milk

Gut dysbiosis such as delayed colonization with bifidobaceria has been well documented in infants delivered by cesarean section, but evidence is emerging on the effects of this surgical intervention on breast milk composition. Mature milk samples, taken from 10 healthy Spanish mothers after 1 month of exclusive breastfeeding, were profiled by Cabrera-Rubio *et al.* with 16S ribosomal ribonucleic acid gene pyrosequencing and targeted quantitative polymerase chain reaction. Despite individual variability in breast milk microbial composition, there was clear separation of the milk microbiome from mothers with a vaginal birth from those who delivered by non-elective cesarean. Relative to vaginal birth, the breast milk microbiota of women delivering by cesarean was less diverse. They contained a greater number of staphylococci and fewer bifidobacteria, and levels of these two microbes were inversely correlated. Hence, breast milk microbiota may have a role to play in the gut dysbiosis of infants associated with cesarean delivery.

Cesarean section delivery lowers the quantity of immunoglobulin A (IgA) in colostrum

IgA is a predominant immunoglobulin present in human breast milk and plays an important role in the maturation of infant gut immunity. Breast milk composition varies between populations and the maternal factors responsible for breast milk IgA concentrations are largely unknown. Munblit *et al.* assayed IgA levels in 294 colostrum samples from the MecMilk International cohort that were collected from women residing in London, Moscow and Verona. IgA levels in colostrum fell with increasing infant age and were lower in Verona than Moscow or London, independent of maternal parity, smoking (which lowered IgA colostrum), fresh fruit and fish consumption, and allergen sensitization. Noteworthy is that following adjustment for these maternal characteristics, colostrum levels of IgA were significantly lower following cesarean section delivery. Hence, cesarean delivery not only disrupts microbial composition of the infant gut and of breast milk, but also has the capacity to directly affect the provision of vital immunity to nursing infants.

Infant fecal IgA levels are influenced by breastfeeding status, maternal parity and infant sex

Initially provided by breast milk, IgA production by the infant gut is gradually stimulated by developing gut microbiota. As shown by Bridgman *et al.* in fecal samples of infants from the Canadian Healthy Infant Longitudinal Development (CHILD) cohort, by 4 months of age, infants can produce considerable amounts of their own IgA in the absence of breastfeeding. Infant fecal IgA concentrations increased with the extent of breastfeeding in male infants. However, they did not vary according to breastfeeding status in female infants. Fecal IgA levels were lower in all infants born to multiparous mothers. Both these results implicate a fetal programming influence on breast milk levels or infant ability to produce IgA.