The Pregnancy Microbiome

Hadar Neuman and Omry Koren

The human microbiome is a collection of bacteria, archaea, fungi, and viruses, all residing within our bodies, including their genetic material. In recent years, research has revealed multiple essential roles of these microorganisms in metabolism, immunity, endocrinology, and overall health. Numerous physiological and disease states, including obesity and the metabolic syndrome, have been correlated with microbial changes, termed dysbiosis. Our microbiomes change in response to our environment, diet, weight, hormones, and other factors. It is, therefore, not surprising that during pregnancy, when dramatic weight gain and metabolic and immune changes occur, there are also significant changes in the microbiome. Throughout pregnancy, alterations in the microbiota composition occur at a variety of body sites, including the gut, vagina, oral cavity, and placenta. Yet, there remains much to be discovered regarding the precise microbial alterations during pregnancy, their timing, and, potentially, their further effects. Understanding the roles of the microbiome throughout pregnancy in health and disease is of great importance for opening new research avenues and suggesting new therapeutic approaches. This includes questions regarding the safety and effects of antibiotics and probiotics during pregnancy on the fetal and offspring health.

The major changes that occur in gut microbiota during pregnancy include an increase in the bacterial load and profound alterations in the composition of the gut microbiota, mostly during late pregnancy [1, 2]. These dramatic changes are characterized by reduced individual richness (alpha diversity), increased between-subject diversity (beta diversity), increased abundance of Actinobacteria and Proteobacteria phyla, and reduced abundance of Faecalibacterium and other short-chain fatty acid producers. Transfer of third-trimester microbiota into germ-free mice was shown to cause increased weight gain, insulin resistance, and a greater inflammatory response compared to the first-trimester microbiota [2]. The vaginal microbiome undergoes significant changes during pregnancy as well [3], including a significant decrease in overall diversity, increased stability, and increased abundance of Lactobacillus species [4].
The main change occurring in the oral microbiota during pregnancy is an increase in the microbial load, including higher levels of the pathogenic bacteria *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, and *Candida* [5]. Antibiotics administered during pregnancy were shown to affect the microbiome composition and diversity, as well as to have some effects on the offspring.

Unlike in the case of disease states, we believe that the microbial alterations observed during pregnancy are vital for a healthy pregnancy. While more research in this field is required to reveal specific mechanisms and pathways regulating these alterations, the microbial changes during pregnancy are likely coordinated with the immune, endocrine, and metabolic states. High progesterone and estrogen levels may affect the microbiome as it has been previously shown that microbial components can respond to and regulate host hormones, and that host hormones influence bacterial growth. Additionally, significant immune changes occur during pregnancy in order to protect the fetus and mother from infection on the one hand, while enabling fetal immune development. These are likely to affect the microbial components as well. Finally, metabolic changes occur during pregnancy, including changes in energy homeostasis, fat storage, and hormonal regulation. Many of these metabolic processes somewhat resemble states of the metabolic syndrome, obesity, and diabetes, which have all been correlated with microbial changes.

Future research will likely reveal the interactions and pathways linking the various physiological changes to the microbial changes, thereby explaining the significance of each change observed. Such studies may also have clinical relevance in terms of recommendations for antibiotic treatments, probiotics, and potential therapies for pregnancy complications.

**References**

Microbial Composition of the Initial Colonization of Newborns

Samuli Rautava

The compositional development of the intestinal microbiota in early life has attracted considerable research interest during the past decade after the association of gut colonization patterns with the risk of noncommunicable diseases has been recognized. To date, intestinal dysbiosis has been linked to conditions ranging from inflammatory or immune-mediated diseases, such as atopic disease, type I diabetes, inflammatory bowel disease, and necrotizing enterocolitis, to overweight and obesity. It is plausible, although not thoroughly proven, that perturbations in gut colonization play a causal role in the development of disease [reviewed in 1]. Epidemiological data demonstrate that aberrant gut microbiota composition in early infancy may precede the development of atopic disease, obesity, infantile colic, or necrotizing enterocolitis. Further corroboration for a causal connection between early gut microecology and the risk of disease has been obtained from both experimental animal models and epidemiological studies linking factors which are known to disrupt early gut colonization, such as cesarean section delivery [2] or early antibiotic exposure [3], to increased disease risk. Elucidating the origin and composition of the intestinal microbiota in the neonatal period and early infancy may, therefore, be of high clinical importance.

Neonates and infants acquire their indigenous gut microbes from other humans and predominantly from the mother. During delivery, the neonate receives a colonizing inoculum of maternal vaginal microbes including *Lactobacillus*, *Prevotella*, and *Sneathia* species [4]. Early colonization patterns of infants born by cesarean section delivery differ significantly from those of vaginally born individuals, and long-term differences in gut microbiota composition, immune development, and disease risk have been reported between subjects born vaginally or by cesarean section [1].

It has recently been suggested that human gut colonization may begin prior to birth [1, 5].
Accumulating evidence suggests that the placenta and amniotic fluid harbor a sparse but distinct microbiota, which may be the source of the microbes detected in meconium. We have recently reported that species belonging to *Bacteroides*, *Lactobacillus*, *Prevotella*, and *Peptostreptococcus* are detectable in both amniotic fluid and meconium [5]. Experimental animal studies have provided evidence to suggest that bacteria introduced in the maternal gut are transported to the amniotic fluid and fetal gut. The details and significance of intrauterine gut colonization remain to be determined.

After birth, the most important modulator of gut colonization is breast milk. Initial neonatal gut microbiota characterized by *Escherichia coli*, enterococci, streptococci, and *Clostridium* species is rapidly followed by anaerobic *Bifidobacterium* and *Bacteroides* species. Breastfed neonates and infants typically harbor an intestinal microbiota dominated by bifidobacteria, which may be detectable already during the first days of life. The predominance of bifidobacteria in breastfed neonates may be explained by human milk oligosaccharides, which selectively promote the growth of specific bifidobacteria and particularly *B. longum* subsp. *infantis*. Interestingly, human milk also contains live microbes, which may colonize the neonatal gut. The neonatal gut microbiota shares features with the maternal milk microbiota already during the first days of life [5], but the significance of the bacteria in human milk to infant health is currently not known.

Disturbances in neonatal gut colonization are often detected as a result of cesarean section delivery, prematurity, antibiotic exposure, or formula feeding. The same exposures have also been associated with increased disease risk, which, therefore, may at least in part be attributable
to intestinal dysbiosis (Fig. 1). Supporting healthy gut colonization by reducing cesarean section rates, prudent use of antibiotics, and promotion of breastfeeding, as well as prebiotic or probiotic supplementation in high-risk individuals, may have a significant impact on child health and deserves rigorous scientific research efforts.

References

3 Turta O, Rautava S: Antibiotics, obesity and the link to microbes – what are we doing to our children? BMC Med 2016;14:57.
Bacterial Colonization of the Newborn Gut, Immune Development, and Prevention of Disease

W. Allan Walker

In the last half century, the disease burden has shifted from a predominately infectious to an immune basis in developed countries [1]. For example, there has been a striking increase in allergic and autoimmune diseases. This shift and increase in diseases has been attributed to the nature of initial bacterial colonization of the newborn intestine, a revision of the so-called “hygiene hypothesis” [2]. During the first 2 years of life, the infant establishes a complete colonization of the gastrointestinal tract that remains as a microbial signature throughout their life (Fig. 1). Normal colonization is dependent on both genetic and environmental factors, particularly the influence of diet on the composition of intestinal microbiota. This observation is particularly true of breast feeding as the initial source of oral feeding [3]. Factors in breast milk affect the composition of intestinal microbiota by stimulating “pioneer” bacteria which are essential in the activation of intestinal immune defenses such as polymeric IgA production. The initial colonization process occurs in stages and has its greatest fluctuations in the early months of life. During the same neonatal period, the newborn infant develops appropriate intestinal host defense mechanisms against infectious and immune-mediated diseases. Since intestinal bacteria influence gut metabolic and immunologic functions, the fluctuations in bacterial colonization at the time when immune homeostasis is developing has profound effects on the infant’s general health and the prevention of disease expression later in life.

An example of this process is the development of immune tolerance (Fig. 2). Immune tolerance to innocuous antigens and commensal bacteria is the absence of a systemic immune response to their stimulus and thus an absence of autoimmune disease states. Oral tolerance develops only with bacterial colonization [4] which also defines appropriate immunologic responses to stimuli, including the nature of the T-helper
cell subset responses and other immunologic cellular subsets [5]. As new studies are reported regarding the association between intestinal colonization and host defense, we have come to appreciate the importance of appropriate initial colonization and immune homeostasis.

Here, we briefly suggest that probiotics have been effective in partially restoring symbiosis to a dysbiotic colonization process. In our studies with breast milk, we have shown that premature infants fed mother’s expressed breast milk compared to formula have a microbiota which favors anti-inflammation over inflammation. This may be an explanation for the protection against necrotizing enterocolitis seen in premature infants given expressed breast milk. Other studies suggest that specific probiotics (e.g., Bifidobacterium infantis) may uniquely inhibit inflammation in premature infants. Probiotics have also been effectively used to reduce the expression of atopic dermatitis of allergy-prone infants when

**Fig. 1.** Schematic representation of a cross section of the small intestine of the human fetus in utero vs. the newborn human infant. The fetal intestine appears thin and exhibits a slow epithelial proliferation rate with a paucity of gut-associated lymphoid-tissue (GALT), whereas the infant intestine manifests a robust, diverse epithelium with a fast turnover rate and abundant GALT elements.
given in the later stages of pregnancy and during lactation in mothers with a history of allergy. A greater understanding of the mechanism of intestinal colonization-induced immune homeostasis in the newborn may allow us to identify routine management of newborns to guarantee health and the prevention of disease.

References


Epigenetics in Gastrointestinal Health and Disease: Spotlight on DNA Methylation in the Intestinal Epithelium

Matthias Zilbauer and Judith Kraiczy

Epigenetics can be defined as stable, potentially heritable changes in the cellular phenotype caused by mechanisms other than alterations in the underlying DNA sequence. DNA methylation is amongst the most intensely studied epigenetic mechanisms known to be operative in mammals and has been shown to play a major role in regulating fundamental aspects of cell biology, including cellular differentiation, organ development, cell type-specific gene expression, and X-chromosome inactivation [1]. Importantly, it is becoming increasingly clear that epigenetic mechanisms, operating at the interface between the genetic code and our environment, are able to mediate environmental changes into stable phenotypic alterations. Given the large body of existing evidence supporting the importance of environmental factors such as diet, nutrition, and exposure to infections and toxins on human health, epigenetic mechanisms provide a plausible mechanistic framework for the development of many multifactorial diseases, including those affecting the gut [2, 3].

The intestinal epithelium as a single cell layer separating the host from its environment has long been recognized as playing a major role in orchestrating homeostasis in the gastrointestinal (GI) tract. The close contact and constant exposure to the external environment including the intestinal microbiome makes it an ideal cell type to investigate the role of epigenetic mechanisms in regulating cellular function during physiological gut development. Moreover, impaired function of the intestinal epithelium has been suggested by many to play an important role in the pathogenesis of inflammatory bowel disease (IBD). However, exact mechanisms responsible for causing malfunction of the intestinal epithelium in humans remain ill defined.

We recently reported on the role of DNA methylation in regulating gene expression and cellular function in the human intestinal epithelium
during physiological GI development by generating genome-wide DNA methylation as well as transcriptional profiles of highly purified human fetal and pediatric intestinal epithelium [4]. Comparisons of these genome-wide signatures allowed us to identify a large number of differentially methylated regions, which also displayed significant changes in their gene expression. Interestingly, many of these regulatory differentially methylated regions were found within genes known to be involved in intestinal epithelial innate defence, e.g., TLR3 (toll-like receptor), PIGR (polymeric immunoglobulin receptor), and MUC2 (mucin). Moreover, performing pathway analysis on regulatory differentially methylated regions revealed a significant enrichment of genes associated with GI and immunological diseases, suggesting that alterations in epigenetic (i.e. DNA methylation) programming during physiological GI development could lead to the development of disease in later life. Indeed, a developmental origin is increasingly being implicated in the pathogenesis of many multifactorial, complex diseases including IBD (Fig. 1). We, therefore, hypothesized that developmentally acquired alterations in the epigenetic profile of the intestinal epithelium could be implicated in IBD
pathogenesis. In order to test this hypothesis, we performed genome-wide DNA methylation profiling of intestinal epithelium obtained from children newly diagnosed with IBD. Strikingly, we observed distinct differences in a subgroup of IBD-derived epithelial samples compared to those obtained from healthy GI mucosa. Importantly, we observed a highly significant overlap between genes undergoing dynamic DNA methylation changes during physiological intestinal epithelial development, i.e., during the transition from human fetal to healthy pediatric epithelium, with those genes displaying altered epigenetic profiles in children diagnosed...
with IBD. Together these findings suggest that alterations in the epigenetic programming of the intestinal epithelium at critical time periods may at least in part contribute to impaired function of this cell layer in patients suffering from IBD. In order to further investigate the functional consequences and underlying mechanisms, we are currently in the process of utilizing novel three-dimensional human intestinal organoid culture models, allowing us to generate “mini-guts” from mucosal biopsies both from healthy individuals and patients diagnosed with IBD (Fig. 2) [5].

References

Gut-Brain Axis and Behavior

Clair R. Martin and Emeran A. Mayer

There has been growing interest in the interactions between the gut microbiome, the brain, and behavior [1, 2]. Even though the interest has been fueled by a series of provocative studies in rodent models, there is growing evidence that some of the reported preclinical findings may translate into human behavior and disorders. Preclinical evidence supports a role of the gut microbiome in behavioral responses associated with pain, emotion, social interactions, and food intake. In humans, the regular intake of a probiotic consortium in healthy women was associated with changes in brain networks involved in emotion recognition [3], and recent studies performed in patients with depression and mouse models generated by fecal microbial transfer from such patients suggest possible causation [4]. On the other hand, data indicating an effect of acute gut microbial alterations induced by probiotics or antibiotics on adult human behavioral and clinical parameters are very limited. Based on currently available data, it is likely that the brain and the gut microbiota are in bidirectional communication. While the reported dysbiotic states in depression, chronic stress, and autism may reflect altered brain signaling to the gut associated with these brain disorders, altered gut microbial signaling back to the brain may play a role in reinforcing brain alterations. On the other hand, diet-induced alterations in the gut microbiome may signal to the brain and alter brain networks involved in ingestive behavior resulting in craving for high fat and sugar intake. A major period of vulnerability and plasticity of brain-gut microbiome interactions occurs during the first few years in life, when both gut microbial systems and brain networks are developing. Plausible explanations for the apparent discrepancy between dramatic results in rodent models and the lack of conclusive evidence for the translatable ability of these findings into human disease populations include the limited homology of the human and mouse brains in terms of brain networks relevant for human brain disorders and the limitations of the gnotobiotic mouse models. Furthermore, there is good evidence that brain-gut microbiome interactions in the adult are fairly stable once they have been established during the first 3 years of life. There are many factors during this developmental period which have been shown to
influence the assembly of the gut microbial architecture, including the diet and stress level of the pregnant mother, the mode of delivery, breast feeding, early adverse life events, and antibiotic exposure. Epigenetic effects occurring during this developmental period could program the nature of brain-gut microbiome interactions for the adult period. In order to overcome the current limitations and uncertainties in this field, there is a need for large-scale, longitudinal human studies in well-phenotyped populations (including pediatric populations) [5]. Such studies should include interventions targeted at the gut microbiome (pre- and probiotics) while monitoring brain and behavioral responses, and at the brain (mind-based therapies) while monitoring the gut microbiota and their metabolites. It is only through these studies that it will be possible to establish causality between brain and gut microbial influences, and to develop novel therapies for human brain disorders aimed primarily at the gut microbiome.

References
Dysbiosis in the Neonatal Period: 
Role of Cesarean Section

Josef Neu

It is very likely that in regions of the world and hospitals with a high cesarean section (C-section) rate many of the C-sections may be unnecessary. Although concern should be raised in regard to the effects of these high C-section rates on the mother, the effect on public health, as well as the health of the individual infant in later life, may be affected by the mode of delivery. With the advent of the human microbiome project in the past decade, it has become increasingly clear that early microbial colonization will have major effects on the individual as they mature. Although the term “dysbiosis” is not clearly defined, it reflects alterations in the normal microbial ecology that may have detrimental health effects on the host. In this manner, early life perturbations of the microbiota (“dysbioses”) are likely to lead to metabolic, immunologic, and epigenetic consequences that have major effects on the developing individual and perhaps may even affect subsequent generations [1].

Recent studies have shown that microbial composition differs in vaginally versus C-section-delivered infants [2]. These differences can be persistent and can be found throughout childhood. Several of the bacteria found in higher quantities in infants delivered vaginally than by C-section are known to have beneficial effects. However, there are confounding factors that lessen the evidence of the microbial differences ascribed to the mode of delivery, i.e., C-section versus vaginal delivery.

However, children born by C-section have an increased susceptibility to several immune-related conditions that are seen in the first year of life. These include asthma, type I diabetes, food allergies, allergic rhinitis, and celiac disease [3]. Other studies have found long-term associations between C-sections and nonimmune-related health outcomes such as increased body mass [4] and effects on the neurological system [5]. Thus, there is a body of epidemiologic literature that demonstrates that the mode of delivery is associated with long-term health outcomes in infants and children. Nevertheless, it is important to remember that these epidemiologic associations do not prove causality.
Confounding factors other than the mode of delivery, such as antibiotic use, maternal stress, diet, timing of the initiation of feedings, and several other factors, need to be accounted for in order to decrease skepticism regarding studies that suggest later immunologic or metabolic diseases are actually influenced by differences microbiota colonization due to the mode of delivery.

From this review, it is obvious that there are strong indications that infants born by elective C-section develop a microbiota that differs from that in babies born by vaginal delivery. This is seen in association with epidemiologic studies that show increased odds ratios of immunologic and metabolic diseases in those babies born by C-section versus vaginal delivery. This may have major public health implications. Studies that tightly control for confounding factors still need to be done. The fact that provision of vaginal bacteria to C-section-delivered babies using a mouth swab may actually transmit these bacteria to the infant of interest, but significant caution needs to be taken, and alternative approaches need to be developed that are safe as well as effective. Follow-up studies showing efficacy as well as safety need to be evaluated in the long term.

References

Early-Life Antibiotic Exposure, Gut Microbiota Development, and Predisposition to Obesity

Meghan B. Azad, Shirin Moossavi, Arthur Owora, and Shadi Sepehri

While antibiotics provide life-saving treatment for infectious disease, they are frequently prescribed inappropriately, especially in young children. Weight gain trajectories appear to be “programmed” in early life, and gut microbiota play a key role in this process [1]. Antibiotics could, therefore, impact the risk of obesity by disrupting the normal establishment of gut microbiota during critical phases of early development (Fig. 1).

Early-Life Antibiotics and Obesity in Humans

Antibiotics have been used as growth promoters in livestock for decades, but this phenomenon had not been studied in humans until recently. Population-based studies have found that antibiotic exposure during infancy (and even in utero) is associated with increased weight gain and obesity risk later in childhood [2, 3]. Some have demonstrated dose-response gradients, with stronger effects from multiple exposures and broad-spectrum antibiotics, although not all studies have confirmed these associations.

Gut Microbiota Development and Obesity

Colonization of the neonatal intestine is critical for neonatal development and has a long-term impact on health. This process begins in utero with the transfer of maternal microbiota via the placenta and continues at birth with the transmission of maternal vaginal and gut microbiota. Once established, the gut microbiota contributes to host weight gain by fermenting indigestible complex carbohydrates and regulating the secretion of gut-derived peptides that impact nutrient absorption, satiety, and energy homeostasis. Disruption of gut microbiota
development early in life could, therefore, foster an “obesogenic” microbiome, as evidenced by predictive microbiota signatures identified among infants who went on to gain excessive weight later in childhood [4].

**Antibiotics and the Developing Gut Microbiota**

Antibiotic treatment during the intrapartum and early postnatal period can influence the developing gut microbiota, with some induced changes persisting up to 1 year after birth [1]. Studies in older infants and children also demonstrate long-lasting effects of early antibiotic exposure, including delayed microbiota maturation and reduced microbiota richness for up to 2 years following exposure. More rapid recovery has been documented with narrow-spectrum versus broad-spectrum antibiotics. Stronger effects are often observed when exposure occurs in the first 6 months of life, suggesting that a critical window exists where antibiotic exposure is particularly damaging to the developing gut microbiota.
Antibiotics, Microbiota, and Obesity in Rodents

Animal models provide the opportunity to precisely control the dose and timing of antibiotic exposures, determine their impact on weight gain, and study the underlying biological mechanisms, including the role of gut microbiota. Cox et al. [5] have characterized the impact of early-life antibiotic exposure in a series of experiments using rodent models, showing changes in gut microbiota maturation and increased adiposity, especially when exposed mice were fed a high-fat diet later in adulthood. Microbiota transplant experiments demonstrated a causal role for microbiota in the obesogenic effect of early-life antibiotics. Interestingly, antibiotic-induced microbiota shifts generally resolved after treatment was terminated, yet the metabolic phenotypes persisted, indicating that microbiota disruption in early life can have a long-term impact on obesity risk, even when the microbiota appears to recover from the perturbation.

Conclusion

Complementary evidence from human and animal research indicates that antibiotic exposure during critical periods of early development may disrupt the normal colonization and alter the “programming” of weight gain trajectories and the development of obesity. Further research is needed to confirm and characterize the causal mechanisms involved. Obesity is clearly a complex and multifactorial condition; thus, a multi-pronged prevention strategy will be required to curb the obesity epidemic. Based on current evidence, this strategy should include the judicious use of antibiotics, especially in early life when the developing gut microbiota is particularly susceptible to perturbations with long-lasting implications for metabolic programming and obesity risk.

References

1 Turta O, Rautava S: Antibiotics, obesity and the link to microbes – what are we doing to our children? BMC Med 2016;14:57.
Necrotizing enterocolitis (NEC) is an acquired inflammatory condition of the gut and carries significant mortality and morbidity in preterm infants with very low birth weight [1–5]. The interplay between toll-like receptors, bacterial endotoxins, the developmentally regulated excessive proinflammatory response of the immature innate immune system, hypoxia, ischemia, reperfusion, free radicals, and presence of substrate is currently thought to play an important role in the pathogenesis of NEC. The association (cause?) of various microbes with NEC has intrigued researchers for many years. The concept of “opportunistic pathogens” suggests that a microbe that is nonpathogenic/nonvirulent in healthy term infants can become pathogenic in an immune-compromised preterm infant.

The association of NEC with a range of bacteria, viruses, and fungal species and the presence of opportunistic pathogens make it difficult to interpret results of the studies assessing early fecal flora in preterm infants. The other factors involved in shaping the early gut microbiota in preterm infants, and the differences in the methodology for its assessment, further complicate the issue. These factors include the mode of delivery, gestational and postnatal age, type of milk feeds, prenatal and postnatal exposure to antibiotics, and postnatal exposure to gastric acid inhibitors. The differences relate to technical issues such as sample processing, choice of the PCR primer, laboratory contamination, and differences in DNA extraction methods. It is important to note that the results of fecal microbiome studies are mostly similar when different methods (e.g. culture-based and molecular methods) are used in the same study. Investigators report that culture-based methods miss relatively few, if any, bacterial species when analyzing the early gut microbiota in preterm infants. Most studies to date have assessed fecal microbiota. However, the results of mucosal biopsies and luminal content analysis seem to suggest or confirm the adequacy of fecal sampling.

The results of recent studies (Table 1) indicate that in contrast to the healthy adult gut microbiota, the early postnatal gut microbiota of
<table>
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<th>First author</th>
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<th>NEC cases, n</th>
<th>Controls, n</th>
<th>Key findings</th>
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<tr>
<td>Warner [6], 2016</td>
<td>16S rRNA, Pyroseq</td>
<td>46</td>
<td>120</td>
<td>NEC was associated with significantly increased Gammaproteobacteria and decreased Negativicutes</td>
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<td>Ward [7], 2016</td>
<td>16S rRNA, DSMSA</td>
<td>27</td>
<td>89</td>
<td>Colonization with uropathogenic <em>Escherichia coli</em> was a risk factor for NEC and subsequent mortality; the strains included ST69, ST73, ST95, ST127, ST131, and ST144</td>
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<tr>
<td>Heida [8], 2016</td>
<td>16S rRNA</td>
<td>11</td>
<td>22</td>
<td>Significantly increased presence and abundance of <em>Clostridium perfringens</em> and <em>Bacteroides dorei</em> in meconium in cases vs. controls; postmeconium samples: abundance of staphylococci negatively associated with NEC; <em>C. perfringens</em> more prevalent in NEC cases</td>
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<td>Hourigan [9], 2016</td>
<td>16S rRNA</td>
<td>1a</td>
<td>1a</td>
<td>Decreased bacterial diversity and increased Proteobacteria in the week preceding the signs of NEC in the twin who developed the illness</td>
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<td>Cortese [10], 2016</td>
<td>16S rRNA, qPCR</td>
<td>–</td>
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<td>Microbial colonization may alter epigenetic signatures of the immature gut establishing inflammatory changes and compromising barrier properties predisposing to NEC</td>
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<td>Abdulkadir [11], 2016</td>
<td>16S rRNA, qPCR</td>
<td>10</td>
<td>10</td>
<td>No significant difference in TBL in cases vs. controls; no significant temporal changes in TBL within cases before vs. after NEC diagnosis, and in controls</td>
</tr>
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<td>Cassir [12], 2015</td>
<td>16S rRNA, Pyroseq, qPCR</td>
<td>15</td>
<td>15</td>
<td><em>Clostridium butyricum</em> specifically associated with NEC using molecular and culture-based methods</td>
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<td>Zhou [13], 2015</td>
<td>16S rRNA</td>
<td>10</td>
<td>26</td>
<td>The specific pathogens (e.g., Gammaproteobacteria) associated with NEC may vary by the infant’s postnatal age at onset of NEC</td>
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<td>McMurtry [14], 2015</td>
<td>16S rRNA, Pyroseq, qPCR</td>
<td>21</td>
<td>74</td>
<td>Microbial diversity and Clostridia abundance and prevalence decreased with increasing severity of NEC</td>
</tr>
<tr>
<td>Raveh-Sadka [15], 2015</td>
<td>16S rRNA</td>
<td>5</td>
<td>5</td>
<td>Strains colonizing each infant in the unit were distinct, and none was common to infants who developed NEC; the paucity of shared gut colonizers suggested significant barriers to the spread of bacteria among infants</td>
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<td>Sim [16], 2015</td>
<td>16S rRNA, FAFLP typing</td>
<td>12</td>
<td>44</td>
<td>A clostridial OTU was overabundant in samples from infants with NEC before diagnosis; culture confirmed C. perfringens type A; FAFLP typing showed that no isolates were identical; samples from NEC cases before diagnosis without profuse C. perfringens showed an overabundance of Klebsiella OTU</td>
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<td>Brower-Sinning [17], 2014</td>
<td>16S rRNA</td>
<td>16</td>
<td>10</td>
<td>TBL was higher in NEC samples; both NEC and non-NEC samples showed high interindividual variability and an abundance of opportunistic pathogens; NEC samples showed an abundance of strict anaerobes and a decreased diversity of the bacterial community and no uniform pattern of microbial colonization</td>
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<td>Claud [18], 2013</td>
<td>16S rRNA</td>
<td>5</td>
<td>5</td>
<td>Gut microbiome differed in cases vs. controls, starting from 3 weeks before diagnosis of NEC; the majority of the differentially abundant genes in cases were associated with carbohydrate metabolism and mapped to the Enterobacteriaceae family</td>
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### Table 1.
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<th>Controls, n</th>
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<tr>
<td>Normann [19], 2013</td>
<td>Barcoded Pyroseq</td>
<td>10</td>
<td>20</td>
<td>Gut microbiota was dominated by <em>Enterococcus</em>, Bacillales, and Enterobacteriaceae in cases; high relative abundance of Bacillales and Enterobacteriaceae preceded the diagnosis of NEC; <em>Enterococcus</em> dominated gut flora in control samples; a low diversity of gut microbiota without significant differences between NEC vs. controls</td>
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<td>Smith [20], 2012</td>
<td>PCR-DGGE</td>
<td>21</td>
<td>142</td>
<td>Gram-positive bacteria predominant in NEC samples; control group had mixed flora of gram-positive and -negative bacteria</td>
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<td>Mai [21], 2011</td>
<td>High-throughput 16S rRNA</td>
<td>9</td>
<td>9</td>
<td>Between the 1-week and &lt;72-h samples, Proteobacteria increased, Firmicutes decreased, and some of the molecular signatures were increased in NEC cases; one of the more frequently detected bacterial signatures matched closest to Gammaproteobacteria</td>
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<td>Wang [22], 2009</td>
<td>16S rRNA, TRFLPA</td>
<td>10</td>
<td>10</td>
<td>Gut microbiota showed low diversity in cases and controls; infants with NEC showed a further decrease in diversity, increased abundance of Gammaproteobacteria, and a decrease in other bacterial species</td>
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The study by Cortese et al. [10] was an experimental study. DSMSA, deep shotgun metagenomic sequence analysis; FAFLP, fluorescent amplified fragment length polymorphism; OTU, operational taxonomic unit; Pyroseq, pyrosequencing; DGGE, denaturing gradient gel electrophoresis; qPCR, quantitative polymerase chain reaction; TBL, total bacterial load; TRFLPA, terminal restriction fragment length polymorphism analysis.

*Pair of twins discordant for NEC.*
preterm infants is simple, very diverse, and dynamic, and plays an important role in the development of NEC. Emerging evidence suggests that microbial colonization may alter epigenetic signatures of the immature gut establishing inflammatory changes and compromising barrier properties predisposing to NEC. Assessing the reproducibility of such findings in large prospective studies with a better study design is important. Further research is required to understand the significance of temporal changes in the gut microbiome in the early postnatal period, specific bacterial molecular signatures, decreased diversity, and the relative abundance of Gammaproteobacteria and paucity of strict anaerobic bacteria that precedes NEC in preterm infants. The possibility that changes in gut microbiota may be a consequence rather than a cause of NEC in preterm infants also needs to be considered.

References

Microbiota and Obesity

Erika Isolauri

Obesity is globally the most prevalent nutritional disorder among children. Two decades ago, the World Health Organization declared obesity a global epidemic [1]. The NCD Risk Factor Collaboration found that in 200 countries and territories the mean age-corrected body mass index has continued to increase in men and women alike, and the risk of becoming obese was higher than that of being underweight between 1975 and 2014. The propagation velocity of the epidemic is high in children and young adults of reproductive age, with the potential to transmit the propensity to the next generation [2]. The risk of escalation of the problem should be given high priority in health policy: prevention is better than cure.

Recent experimental and clinical data provide one new target for interventions aiming to reduce the risk of obesity: the microbiota. Scientific interest in the potential causative role of the gut microbiota in obesity was attracted by the demonstration that a distinctive gut microbiota composition prevails in obese individuals, with adjustments following weight gain or weight loss [3]. Aberrant compositional development of the gut microbiota is documented during breastfeeding in infants in whom overweight development was documented, i.e., gut microbiota deviation precedes obesity [4]. Further support for the conception of microbiota involvement in obesity is obtained by epidemiological data linking known causes of gut microbiota disturbance early in life, namely cesarean section delivery and antibiotic exposure, to the subsequent development of overweight and obesity [reviewed in 2]. Experimental studies, again, have improved our understanding of mechanisms and causality in this context [5]. The Western diet with its high fat and energy content has been associated with reduced gut microbiota diversity and perturbed composition, dysbiosis, an imbalance in the taxonomic composition of the gut microbiota. The gut microbiota impacts on metabolism by retrieving nutrients otherwise inaccessible to the host (Fig. 1). Over and above processing nutrients and regulating their access to and storage in the body, activated inflammatory pathways are a corollary to “obesogenic microbiota.”
Indeed, the chronic low-grade inflammation of obesity may be initiated in the gut, and specifically by the gut microbiota, the presence of which is a prerequisite in the progression.

Taken together, these elements markedly reinforce the hypothesis that modification of gut microbial communities might offer a strategy applicable to obesity management and risk reduction. Initially, the hypothesis calls for a prudent review of clinical practice, which may interfere in the healthy host-microbe interaction during the critical period within which, according to the developmental origins of the health and disease theory, the immune and metabolic phenotype is consolidated. Further, the contribution of the mother’s health, weight, and weight gain during pregnancy to the metabolic health and disease risk of the child is recognized as being to a degree determined by the maternal gut and breast milk microbiota [6]. One attractive idea, arising from the extended hygiene hypothesis linked to the developmental origins of the health and disease theory, is modification of the maternal gut microbiota during pregnancy and the perinatal period, as well as the breast milk microbiota. Promoting
a balanced host-microbe interaction may aid in attuning the child's gut microbiota to age and reprogramming the risk of chronic inflammatory conditions, including obesity.

The ultimate goal of clinical research in this context is defining an age-specific gut microbiota endorsing healthy development, documented in children remaining healthy also in the long term and thereby providing a gold standard of healthy human microbiota in the environment studied. Ultimately, it is then the task of clinical intervention studies, in well-characterized human populations, to provide the final proof of causality for nutritional recommendations to combat the obesity epidemic.

References

Microbiota in Functional Intestinal Disorders of Infancy: Implications for Management

Thomas R. Abrahamsson, Richard Y. Wu, and Philip M. Sherman

The complex and diverse intestinal bacterial microflora is now recognized as important in promoting human health. An altered gut microflora, referred to as dysbiosis, is increasingly recognized as having an etiologic role in a variety of conditions, including functional gastrointestinal disorders: colic in infants and irritable bowel syndrome in older children and adults. Probiotics are defined as live microorganisms that, when ingested in sufficient amounts, restore microbial homeostasis and have a benefit on health. Randomized controlled trials indicate that certain probiotics are effective in a variety of intestinal conditions, including functional gastrointestinal disorders such as colic and irritable bowel syndrome.

By Rome IV criteria, colic is the manifestations of recurrent (>3 times/week) and protracted (>3 h/day) periods of irritability, crying, and fussiness that begins in otherwise healthy babies during the first few months of life [1]. Reduced diversity in bacterial species was identified in fecal samples taken at 2 weeks of age from 12 infants who went on to develop colic versus age-matched controls [2]. In particular, the numbers of bifidobacteria and lactobacilli were reduced in infants who went on to develop colic. Randomized controlled trials indicate that *Lactobacillus reuteri*, strain DSM 17928, provided at a dose of $1 \times 10^8$ bacteria, delivered as 5 drops once daily, for 3–4 weeks, is more effective than placebo in alleviating symptoms of fussiness and irritability in colicky babies (Table 1). These prospective clinical studies were undertaken in Italy, Poland, Australia, the People's Republic of China, and Canada. Meta-analysis indicates that the active agent was more effective than placebo, with 5 of the 6 studies included in the evaluation having an impact that reached statistical significance [3]. It should also be noted that even though the total number of infants entered into all of the clinical trials is relatively small, there have been no serious adverse events reported.
Strain-specific effects on colonization resistance, epithelial barrier integrity, modulation of signal transduction events, impacts on innate and adaptive immune responses, and effects on visceral hyperalgesia likely explain the observed variability between strains. In the future, probiotics are likely to be chosen for use in a defined clinical setting based on underlying mechanism(s) of action. The precise component of the probiotic agent mediating the observed effects is the subject of current research. In addition, there is the possibility of developing specific, disease-targeted designer probiotics.

Dose-escalation studies and head-to-head comparisons with other probiotic strains and mixtures of strains are warranted. Whether such interventions early in life will change the composition of the gut microbiome and impact mucosal immune functions and sensations of visceral pain in the long term requires ongoing study and the continued surveillance of those subjects already entered into clinical trials (Table 1).

### Table 1. Probiotic Lactobacillus reuteri DSM 17938 versus placebo in the management of infants with colic

<table>
<thead>
<tr>
<th>First author</th>
<th>Country</th>
<th>Duration, days</th>
<th>Change in crying duration, min/day</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Savino [4]</td>
<td>Italy</td>
<td>28</td>
<td>−66.8</td>
<td>−78.4, −55.2</td>
</tr>
<tr>
<td>Savino [5]</td>
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<td>−125.0</td>
<td>−172.0, −78.0</td>
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<td>Poland</td>
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<td>−55.2</td>
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<tr>
<td>Sung [7]</td>
<td>Australia</td>
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<td>−11.0</td>
<td>−78.6, 100.6</td>
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<tr>
<td>Mi [8]</td>
<td>China</td>
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<td>−55.1</td>
<td>−55.1, −50.9</td>
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<tr>
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<tr>
<td><strong>Summary</strong></td>
<td></td>
<td></td>
<td>−55.9</td>
<td>−64.4, −47.3**</td>
</tr>
</tbody>
</table>

**p < 0.001. 1 Adapted from: Harb et al. [3].

References

The trillions of microorganisms that reside in our gastrointestinal tract known as the gut microbiota play an important role in our health. Throughout life, the gut microbiota is exposed to various substances, including diet, antibiotics, and xenobiotics, that continually shape its structure and function. The development of a diverse and stable gut microbiota is crucial to various host physiologic functions, including immunoregulation, pathogen prevention, energy harvest, and metabolism. As a shared substrate between the host and the gut microbiota, diet exerts strong influences on both. Diet impacts the health of the host not only through a direct nutritional effect but also via the gut microbiota through microbial metabolite production (Fig. 1). For example, bacterial fermentation of dietary complex carbohydrates produces short-chain fatty acids such as acetate, butyrate, and propionate. Butyrate serves as an important energy substrate for the colonic epithelium, whereas acetate and propionate can serve as substrates for lipogenesis and gluconeogenesis as well as regulate different gene expressions by binding to G protein-coupled receptors GPR41 and GPR43 [1]. At the same time, diet can lead to host disease states through microbial metabolite production. Bacterial metabolism of dietary choline produces trimethylamine (TMA) via TMA lyase. TMA is further metabolized in the liver to produce TMA-N-oxide, which has been associated with the development of atherosclerosis and cardiovascular disease [2]. One important consideration is that the equilibrium state of the host plasma metabolome involves both input from diet and microbial metabolite production and output via urinary excretion. Accumulation of toxic metabolites in renal insufficiency may be linked to disease, as TMA and TMA-N-oxide levels were found to be elevated in patients with end-stage renal disease [3], and age-adjusted risks of death and cardiovascular events vary inversely with estimated glomerular filtration rate [4]. These findings suggest that metabolomic profiling of patients with chronic kidney disease versus healthy controls may provide additional insight into the critical link between dietary intake, microbial metabolite production,
and disease. Inflammatory bowel disease (IBD) is a group of inflammatory conditions primarily involving the gastrointestinal tract; IBD is associated with a dysbiotic gut microbiota characterized by a decrease in species richness and the outgrowth of pathogenic bacterial taxa [5]. It is unclear if intestinal inflammation leads to the development of dysbiosis,
or dysbiosis perpetuates intestinal inflammation. The pathogenesis of IBD remains complex and involves immunodysregulation in a genetically predisposed individual to triggers such as food and/or the gut microbiota (Fig. 2). Modulation of the latter two may represent important strategies to treat IBD. Fecal microbiota transplantation using stool from healthy donors to reverse dysbiosis has shown variable success in treating IBD. However, the use of defined formula diets has shown benefits in inducing remission in IBD either alone or in conjunction with immunosuppressive medications. The exact mechanisms by which defined formula diets ameliorate IBD remain unclear and may include exclusion of or reduction in luminal antigens derived from food or changes to the gut microbiota [5]. This illustrates the complex and integral relationship between diet, the gut microbiota, and the host that entails further investigations for health maintenance as well as disease prevention and treatment.

References

Enzymes in Human Milk

David Dallas and J. Bruce German

The term ‘milk’ does not do justice to the complexity, biological activity, or health value of mammalian lactation. Milk is active, dynamic, personal, and alive. Annotating milk components with the functions of those changes requires new models, new experimental tools, and a new vision for milk as an entire system. Milk and infants are constantly changing during lactation. This time dimension has not been fully appreciated, especially how closely milk activities and infant maturity are coordinated.

New tools are examining human milk proteins as they are digested within the infant stomach [1]. Hundreds of novel peptides have emerged [2].

Figure 1 graphically documents that ‘native’ milk, i.e., human milk samples prior to consumption by the infant, contain a diverse array of peptides, and that peptide diversity and abundance rises rapidly within the infant stomach.

Proteolysis within the mammary gland [3] and within the infant shows considerable protein selectivity and linkage specificity. That specificity of proteolytic cleavage is solvable by new computational strategies [2] that tentatively identify the enzymes that are responsible [4]. Enzymes are not from the infant but rather from the milk itself. Transcriptional profiling shows that the enzymes predicted by computational analyses of peptides were actively expressed as mRNA in the mammary gland [5]. Milk is thus a mixture of proteases, zymogens (protease precursors), and protease activators and inhibitors, including components of various proteolytic systems such as plasmin, cathepsin, elastase, kallikrein, and amino and carboxypeptidase systems [4]. The mechanisms by which enzymes reach milk within the mammary gland are shown in Figure 2.

Milk needs to be understood for its dynamic nature, its diversity, and its biological activities. Specific peptides are released at specific times into specific sites along the mammary gland and infant intestinal tract. This is possible by virtue of the protein structures in milk, the enzymes that are present, and the ability of the infant to activate those enzymes at specific
Figure 1. Ion abundances in intact human milk and gastric aspirates from human infants 1 h after consuming the same human milk.

Figure 2. Schematic of the production and transfer of enzymes into milk within the lactating mammary gland.

places and times [6]. Understanding this magnificent system of nourishment is likely to provide insights for nourishing humans of all ages and all health conditions.

References


In recent years, there has been a tremendous increase in our knowledge regarding specific effects of human milk oligosaccharides (HMOs) [1]. Even the first human studies with infant formula supplemented with single HMOs have already been published [2, 3]. To decide which compound(s) would be most suitable for supplementation, in which concentrations or combinations, and how long they should be given, studies are needed regarding the metabolic fate of HMOs as well as their local and systemic effects.

As human milk contains 5–15 g/L of HMOs, large amounts of oligosaccharides reach the gastrointestinal tract of a breastfed infant (Fig. 1). About 1–5% of HMOs seem to be excreted via the infants’ urine [4–6]. Determining individual HMOs in urine samples, we found that, for example, about 50–160 mg LNT or lacto-N-fucopentaose (LNFP) II can be detected. Hence, several hundred milligrams per day circulate in the infant’s blood. Therefore, it can be expected that HMOs not only exert local effects within the gastrointestinal tract but also systemic effects.

A straightforward strategy to investigate HMO metabolism is to determine the presence or absence of natural milk oligosaccharides in feces and urine. However, the high structural diversity of oligosaccharides in human milk complicates acquiring a sophisticated knowledge of “what structures are important for infant health” despite recent methodological developments to characterize minute amounts of HMOs and their degradation products in biological samples.

As recent data support the hypothesis that there is a link between the Lewis blood group and secretor status of an individual and certain inflammatory diseases, this review will focus on the metabolic fate of secretor/nonsecretor- and Lewis blood group-specific components.

We conclude that there is no simple urinary or fecal excretion pattern of HMOs, although the pattern in urine often reflects the
mother’s secretor/nonsecretor status. However, there are deviations for single HMOs which deserve special attention. In feces, the variation in excretion is much higher than in urine, which may be caused by variations in the infant’s intestinal microbial composition. There is no gradual decrease in the HMO excretion with time as proposed earlier, as even after 7 months of exclusive breastfeeding intact HMOs can be detected in some infants.

In addition, we found that whenever oligosaccharides were detected in feces, LNT, the major core structure of HMO, was present. Hence, our data do not support speculations that LNT is a preferable source for the microbiota.

Data presented so far raise the question of whether human milk could be used as the gold standard with regard to a reference for the composition of HMOs. There are large differences in the total amount and in the pattern of individual HMOs based on the genetic background of the mother. Is the quality of human milk minor when specific HMOs are missing as is the case in nonsecretors, which comprise about 20% of the population? Or do infants fed “secretor”-specific milk deserve special attention assuming that they may have a higher risk for developing...
certain diseases? In order to provide answers to these questions, it may not only be necessary to identify the HMOs with the highest benefit for most infants but also to differentiate according to the health risk of infants influenced by their maturity at birth as well as their own genetic background.

References
Breastfeeding benefits human infants in numerous ways, e.g., by influencing the infant's intestinal microbiome. The composition of an infant's gut microbiome can impact their immediate and future health. Bifidobacteria play a major role in structuring the gut microbiome of breastfed infants due to their ability to consume oligosaccharides found in human milk. However, recent studies have revealed that bifidobacteria are often missing from the gut microbiome of many breastfed infants in some locations. This lack of colonization may be due either to (1) differences in the environmental conditions in the gastrointestinal tract of uncolonized infants which prohibit the growth of bifidobacteria or (2) a dearth of sources from which infants may acquire these specialized bacterial species.

Several differences in ambient environmental conditions in the gut between infants in different locations may bar bifidobacterial colonization. Breast milk has been the principal source of nutrition for infants over human evolutionary history, and it is known that formula feeding leads to disruptions in the typical pattern of microbiota development in infants [1]. Milk contains macronutrient concentrations of oligosaccharides and glycoconjugates which pass undigested through the infant small intestine and act as prebiotics supporting the establishment of bifidobacteria [2]. It is plausible that cultural differences in the duration or rates of breastfeeding between locations may lead to differential colonization by bifidobacteria due to the availability of these selective growth substrates. Antimicrobial use is another environmental condition that may influence the colonization of infants by bifidobacteria.

Routes of colonization by bifidobacteria which may be disrupted include maternal transfer via vaginal birth, fecal-oral routes, or via breast milk itself. Transfer from the mother's vaginal canal during birth is the typical first major source of inoculum [3]. Cesarean section birth
limits exposure to possible inoculation both via maternal stool during birth and vaginal contact. Infants born by cesarean section often possess distinct gut microbial communities which are occasionally lower in bifidobacteria [1]. Caretaker skin, siblings, pets, and the built environment may also be vectors for the early transfer of intestinal microbes [4].

Breast milk contains microbes, including bifidobacteria, but their origin and potential impact on colonization is unclear [5]. A so-called "enteromammary" pathway has been postulated whereby the mother's immune system gathers microbes from the mother's gastrointestinal tract and, without killing them, transfers them to be expressed from the mammary gland to the infant during suckling at the breast [5]. This hypothesis remains speculative. Whether an elaborate system for obtaining what were likely common infant intestinal microbes would be advantageous in the environment of evolutionary adaptedness or whether a simple fecal-oral route would suffice is an open question. A careful contemplation of the conditions experienced by bifidobacteria over human evolutionary history may lead to further hypotheses as to the causative factors of comparative undercolonization by this foundation species in some contemporary locations.

The past century has seen drastic shifts in both the selective pressures experienced by the human gut microbiota and the opportunities for transmission of microbes. The possibility for undesirable side effects of these changes has become increasingly clear. Lack of exposure to commensal species such as bifidobacteria was unlikely to be an issue often faced by our ancestors. Human physiology may "expect" bifidobacterial presence during the life stages concurrent with breastfeeding. Bifidobacteria may provide important developmental cues and protection from disease. The disappearance of these exposures may lead to sequelae of public health importance. While further study is needed on the importance of bifidobacterial colonization to infants from diverse contexts, given the apparent benefits of their presence, identifying the cause(s) of this phenomenon and developing solutions is of interest.

References


Human milk oligosaccharides (HMOs) are a group of sugars which are structurally diverse unconjugated glycans. As ingested food components, they are found in human milk with a composition unique to each lactating mother. They have not been used in any other food previously. Therefore, the application of HMOs to neonatal and other food supply requires both safety assessment as well as the assessment of potential health benefits on later life.

In the majority of countries, HMOs are characterized as novel foods in food regulation. Consequently, a mandatory safety assessment and, subsequently, also an efficacy assessment is required.

Issues pertaining to novel HMOs have increased rapidly due to the fast-paced research on the health impact of HMOs and the possibility of large-scale production. HMOs may represent novel tools to modulate the gut microbiota of infants, children, and adults potentially improving health outcomes. Such food ingredients are in demand as the importance of the gut microbiota on health has been revealed. HMOs could be regarded as prebiotics, i.e., components that promote health by influencing the composition and activity of gut microbiota. Therefore, they are specific components targeted toward unique outcomes and functionalities promoting health.

**Regulatory Framework**

The regulations governing the introduction of novel foods vary by geographical region. In some cases, confusion can result in differentiating novel foods from functional foods. In the European Union, for example, the regulatory category of functional foods is formed by foods with European Commission-approved health claims. The fundamental difference between these 2 categories of foods is that novel foods must be evaluated based on their safety, whereas foods with health claims need to be evaluated for any desired nutritional, functional,
or disease-related risk reduction properties of either the food or specific components in the food. Figure 1 demonstrates that the terms are distinct, but sometimes foods and food ingredients fall in both categories, which then necessitate evaluation for both safety and efficacy. The 2-category evaluation in Europe appears most likely for HMOs [1–3].

As novel prebiotics, HMOs can potentially be components of conventional foods, food supplements, or foods for particular nutritional uses. Foods incorporating novel ingredients comprise also those designed for specific dietary requirements and may include infant formulas and follow-on formulas, processed cereal-based food, and food for special medical purposes, as well as total diet replacement for weight control. Until now, 2 HMOs have been assessed for safety in the European Union, and 3 components have been submitted for notification as generally recognized as safe in the USA.

**Health Benefits of Human Milk Oligosaccharides**

As health benefits are expected for HMOs, claims on human health are likely to be submitted. They may include, for example, reducing the number of pathogens in the gastrointestinal tract to support healthy intestinal tract and bowel movements, and claims of efficacy require detailed analysis of benefits and documentation as well as assessment of regulatory bodies. No health claims have been approved for HMOs as of yet. However, several studies suggest that health benefits can be documented and that future health claims may be possible.
References


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