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Faculty Co-Chairs

J. Bruce German, PhD
Professor of Food Science and Technology and Director of the Foods for Health Institute
University of California – Davis
Davis, California, USA

William D. Rhine, MD
Professor of Pediatrics (Neonatology)
Stanford University School of Medicine
Palo Alto, California, USA
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Clinical and basic science research have identified a number of important biological effects of breastfeeding and an exclusively human milk diet on the nutrition and development of infants, particularly preterm and low birth weight infants. Despite this growing recognition, our understanding of the components of human milk, their functions, and their specific clinical implications remains incomplete. Therefore, the properties and potential clinical uses of human milk continue to be the focus of numerous investigations.

The program for the 4th Annual International Conference on Human Milk Science and Innovation (ICHMSI), which is sponsored by Prolacta Bioscience, Inc., included presentations from international experts in a variety of scientific fields on a wide breadth of topics related to breastfeeding, the clinical use of human milk, and scientific research on the components of human milk. The scope of the 4th ICHMSI highlights both the integrative nature of human milk research and its potentially broad clinical applications and impacts.

The biology and science portion of the conference focused on microbiology aspects, including implications for the use of a human milk diet on the establishment of the infant gut microbiome and the development of a novel antibiotic based on the antimicrobial properties of lactoferrin, a well-known component of human milk. A mini-symposium included discussion about human breast milk as a critical determinant of biological diversity from the perspectives of a clinical physiologist and an evolutionary biologist. The clinical aspects portion of the conference included presentations on improving breastfeeding outcomes; clinical methods, outcomes, and implications for the use of an exclusively human milk diet in preterm infants; the use of omic technologies to better understand the mechanisms behind the effects of enteral feeding on the gut of preterm infants; and investigation of the biological relationships between obesity, diet, and lactation.

Highlights from the 4th ICHMSI are captured in these proceedings. It is our hope that continued discussions from meetings such as the 4th ICHMSI will help to further increase awareness of the needs of preterm and low birth weight infants, and the unique clinical benefits provided by an exclusively human milk diet.
Mother’s milk has sustained mankind since the very beginning of recorded human history, when Eve (as in Adam and Eve) breastfed her children. The production of milk is unique to mammals, who take their name from the Latin word “mammalis,” or the “breast,” and each species of mammals is specialized to make milk that is perfect for its offspring. Humans are no exception. However, once scientists began taking an interest in breastfeeding, the focus actually shifted away from breastfeeding and human milk.

Women initially turned to wet nursing to nourish their infants out of necessity, as childbirth was often a risky business. Eventually, wet nursing became a sign of prestige and its use increased; however, there were some concerns around its safety. Wet nursing was prevalent enough in 1800 BCE that Hammurabi’s Code was developed to set forth regulations for wet nursing, including the requirement for a woman to be of high standing and good reputation in order to be a wet nurse. During Spartan times, the wife of the king was required to nurse her eldest son; if not breastfed, the oldest son could not become king. Hippocrates was quoted as saying that “one’s own milk is beneficial, others’ harmful.” In 1662 CE, the Dowager Countess of Lincoln wrote on the importance of breastfeeding one’s infants to improve their survival. Still, throughout the 1700s wealthy women, particularly those in Europe, continued to give their infants to wet nurses. The reign of Marie Antoinette, in particular, was notorious for the automatic use of wet nursing, which resulted in laws to control the practice: a woman had to register as a wet nurse, could not nurse more than 2 infants (plus her own), and must have a separate crib for the infant.

Medical minds then began to look to substitutes for human milk altogether. Foundling homes during the 18th century commonly fed infants gruel made from bread grain mixed with either water or milk from another species; however, the death rate was near 100%. As infants progressively became under the care of physicians instead of only their mothers, physicians began research formulations that could replace the wet nurse. To do so, they looked to other mammalian species (Table 1). Many early formulations consisted of cow’s milk mixed with sugar and water, although scientists also researched the use of milk from goats, horses, and donkeys. As the concept of alternatives became more common, 19th-century physicians such as Dr. William H. Cumming of Atlanta and Dr. A.V. Meigs of Philadelphia sought to create formulations that more closely mimicked human milk by also adding cream for fat and lime water to adjust the pH in order to improve digestion. By the 1870s, Henri Nestle was marketing his infant formula—made of “special” (Swiss) cow’s milk mixed with sugar and modified wheat flour—to mothers in Europe and America. Shortly thereafter, The Ladies’ Home Journal, a key

### Table 1. Constituents of species’ milk.

<table>
<thead>
<tr>
<th></th>
<th>Total solids %</th>
<th>Fat %</th>
<th>Protein %</th>
<th>Carbohydrates %</th>
<th>Ash (residual after burning) %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>12.4</td>
<td>3.8</td>
<td>1.0</td>
<td>7.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Donkey</td>
<td>11.7</td>
<td>1.4</td>
<td>2.0</td>
<td>7.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Cow</td>
<td>12.7</td>
<td>3.7</td>
<td>3.7</td>
<td>4.8</td>
<td>0.7</td>
</tr>
</tbody>
</table>
reference for wealthy and/or well-educated women, also began promoting the use of commercialized infant formulas. The 1920s constituted another period of social “dazzle,” when breastfeeding was considered unfashionable, and the use of infant formula was common until the Great Depression.

Despite the commercial and societal emphasis on formula feeding, research from as early as 1922 showed that survival was much greater throughout the first year of life for infants who were breastfed, even among those who were only partially breastfed (Figure 1). So, for decades the scientists went back to their laboratories to further study potential substitutes, mainly based on cow’s milk and soy; however, their search focused on the most practical substitute for human milk, not the closest or best. Little effort was given to the science of human milk. Mothers were assaulted by formula companies that were trying to convince them that their products represented an improvement over breast milk.

Generation after generation, the search for a replacement for mother’s precious gift of time and milk was sought by the well-educated woman. It was the well-educated woman who led the march to the bottle. The bottle meant freedom. It was then the well-educated woman who led the march back to the breast in the 1950s and 1960s when she realized what had been lost. Scientists finally began to look at human milk, recording its impact on the growth and development of the breastfed infant and exploring the properties that make human milk so gentle on the infant gut and protective against infection, chronic disease, and allergy (Figure 2). The World Health Organization and UNICEF spoke up and, in 1990, nations came together to develop the Innocenti Declaration: On the Protection, Promotion, and Support of Breastfeeding.

To date, little has still been done to understand why some women cannot make enough milk for their babies, or why some women make low-calorie milk and others make high-calorie milk. We have not unraveled the mystery of why some milk turns sour on storing. Nor is there agreement on what is the safest way to provide human milk for all infants. It is hoped that ongoing research on the properties and use of human milk will provide answers to these and other important questions.

Figure 1. Death rates by method of feeding. 

![Figure 1](image1.png)

Figure 2. Bioactive components of human milk.

![Figure 2](image2.png)

Reference

Biology & Science of Human Milk

Mom’s Milk and Microbes

Grace M. Aldrovandi, MD, CM
Professor of Pediatrics, David Geffen School of Medicine, University of California – Los Angeles; and
Chief of the Division of Infectious Diseases, Mattel Children’s Hospital
Los Angeles, California, USA

It is recognized that breast feeding plays an important role not only as a source of nutrients to drive infant development, but also in immune protection and the establishment of the infant microbiome, which shapes the infant immune system. However, for some mothers, such as those with HIV infection, the issue of breastfeeding is more complicated due to concerns over the risk of infant acquisition of HIV. In resource-rich areas, the risks of potential HIV infection are viewed by public health authorities as outweighing the benefits of breastfeeding, and HIV+ women are steered toward alternate options (eg, donor human milk). However, in resource-constrained areas, the considerable infant morbidity and mortality associated with not breastfeeding coupled with the effectiveness of antiretrovirals in preventing HIV transmission has led to the recommendation that HIV-infected women breastfeed their infants. This dichotomy highlights the need to better understand the role and implications of breastfeeding in the context of maternal HIV.

Balancing the Risk of Infant Exposure to HIV During Breastfeeding

Early weaning of infants may reduce the risk of HIV transmission through breast milk. However, in less developed areas of the world, breastfeeding remains an important source of nutrition and immune protection for infants, and early weaning is associated with considerable infant morbidity and mortality.

A randomized clinical trial (ZEBS) of approximately 1,000 HIV+ mothers in Lusaka, Zambia evaluated the effect of exclusive breastfeeding for 4 months (actual median duration of 4.5 months) followed by rapid weaning or breastfeeding for 6 months followed by the introduction of complementary foods and continued breastfeeding per the mother’s choice (median duration of 16.2 months); single-dose nevirapine was provided to prevent HIV transmission. The study endpoint was infant HIV-free survival (HIV infection or death). The study found that early weaning decreased the risk of HIV transmission; however, this was offset by increased infant death, so there was no difference in HIV survival at 24 months between the 2 groups.1 Additionally, early weaning of infants who were HIV infected by 4 months was associated with a higher infant mortality rate for those whose mothers had been assigned to the early weaning group compared with infants whose mothers had been assigned to continued breastfeeding (73.6% vs 54.8%; \( P = 0.007 \)).

Early weaning was associated with a 1.8-fold increase in the rate of diarrheal episodes and a 3.8-fold increase in the rate of diarrhea-associated hospitalization or death between Months 4 and 6.2 Among uninfected children born to HIV+ mothers, early weaning was associated with a significant increase in the risk of infant mortality (Table 1).3 Overall, analyses from the ZEBS study showed that there was no survival benefit to early weaning. The risk of HIV acquisition was similar to the risk of dying from infectious diseases. With the use of antiretroviral therapy, the risk of maternal transmission of HIV during breastfeeding is significantly reduced, further tipping the balance in favor of continued breastfeeding. The cost of antiretroviral therapy is also much less than the cost of formula, making continued breastfeeding a cost-effective option as well.
Table 1. Impact of early weaning on uninfected infant mortality.

| Weaned at age shown vs weaned at >18 months of age, hazard ratio (95% CI) | Age when breastfeeding stopped |
|---|---|---|---|---|
| | 0-3 months | 4-5 months | 6-11 months | 12-18 months |
| 3.59 (1.69-7.62) | 2.03 (1.13-3.65) | 3.54 (1.68-7.46) | 4.22 (1.59-11.24) |

Impact of Maternal Disease Severity on Breastfeeding Outcome

Subanalyses from the ZEBS study found that uninfected infants born to HIV+ mothers with advanced disease were at high risk of mortality and morbidity during the first few months of life even without postnatal transmission of HIV through breastfeeding. Uninfected infants of mothers with low CD4+ T cell counts (<350 cells/µL) were more likely to be hospitalized (HR = 2.28 [95% CI, 1.17-4.45]) or to die (HR = 2.87 [95% CI, 1.03-8.03]). Most hospital admissions were for pneumonia and/or sepsis. Nearly half (47.6%) of infants died if their mother died within 4 months after giving birth, compared with just 3.4% of infants whose mother survived. The cause and effect of the relationship between more advanced disease and poorer infant outcome is not completely understood, but has been replicated in many studies in Africa.

Among mothers who had severe disease (eligible for antiretroviral therapy) but did not receive the therapy, weaning before 15 months was associated with better infant outcomes ($P = 0.002$) because these women were more likely to transmit HIV to their infants. In contrast, among relatively healthy mothers with less advanced disease, weaning before 15 months was associated with a 3.34-fold increase in the risk of HIV infection or death ($P = 0.003$). These findings are not due to a difference in nutrition, as all of the families in the study were getting food. Similar results have been reported from studies in other parts of the world.

Natural Protection Against HIV Transmission in Breast Milk

An analysis of the ZEBS study found that the risk of postnatal HIV transmission from infected mother to uninfected infant during the first 4 months more than doubles with partial (non exclusive) breastfeeding versus exclusive breastfeeding ($P = 0.004$). This finding was irrespective of the mother’s severity of disease, CD4 count, plasma viral load, and syphilis screening results, as well as low birth weight of the infant. The higher postnatal transition rate seen with partial breastfeeding is likely due in part to an influx of virus in the milk as a result of the increased mammary epithelial permeability associated with infrequent suckling.

Researchers have tried to understand the roles of different human milk constituents and their contributions to (or protection from) the transfer of HIV to infants, but it is a complicated process. Animal studies demonstrated that human milk has a unique and potent species-specific ability to inhibit HIV activity and prevent multiple routes of infection, with these properties likely due to the cumulative effect of multiple human milk constituents.

One major difference between human milk and infant formula is the presence of human milk oligosaccharides (HMOs). HMOs are non-digestible by humans, but about 10% of the energy required to produce milk is spent in the production of HMOs. One of the primary purposes of providing HMOs is nutrition to the infant microbiome. Among HIV-infected women, total HMO concentrations above the median (1.87 g/L) conferred a reduced risk of HIV transmission via breastfeeding ($P = 0.04$, after adjustment for CD4+ T cell count and breast milk HIV RNA concentration). Higher breast...
milk concentrations of HMOs were also associated with significantly reduced mortality among HIV-exposed, uninfected infants during breastfeeding. Interestingly, breastfeeding was only protective against mortality in uninfected infants with high concentrations of fucosylated HMOs, suggesting an important role for HMOs in protection against postnatal HIV transmission.

HIV-exposed, uninfected infants were shown to have significantly different and potentially dysbiotic microbiomes compared with unexposed infants despite similar gut microbiomes and breast milk bacterial communities in mothers with and without HIV infection. Small differences in the mothers’ microbiomes may have a direct impact on their infants’ microbiomes. However, the observed differences in the infants’ microbiomes were found to also be due to changes in the HMO content of the mothers’ milk. The relative abundance of 3-sialyllactose, 3-fucosyllactose (3FL), and 2’-fucosyllactose in human milk tended to be higher in HIV+ mothers, while lacto-N-tetraose (LNT) and lacto-N-neotetraose tended to be lower (Figure 1).

Correlations were seen between specific HMOs in the breast milk and bacteria, and the correlations were different between mothers with and without HIV. Thus, maternal HIV infection was found to perturb the development of the infant’s gut microbiome, which can also lead to disrupted development of the infant’s immune system, thereby explaining part of the higher morbidity and mortality seen among HIV-exposed, uninfected infants compared with unexposed infants.

Several other constituents of human milk have also demonstrated a role in limiting the transmission of HIV and/or inhibiting HIV activity or infection, including HIV-specific CD8+ cells, toll-like receptor 2, interleukin-7, and interleukin-8. However, much remains unknown about the roles of specific human milk constituents in minimizing HIV transmission and infection, or about how these constituents interact with the microbial communities to influence the infant’s immune and metabolic programming.
References


Monitoring and Manipulating the Intestinal Microbiome – Lessons From IBD

Jonathan Braun, MD, PhD
Professor and Chair of Pathology and Laboratory Medicine, David Geffen School of Medicine,
University of California – Los Angeles
Los Angeles, California, USA

The human intestinal microbiome varies greatly among individuals, with each individual carrying only a subset of the >1,000 microbial species that can be found in human feces. The abundance of shared microbes also varies greatly between individuals. Further, microbial composition is now known to differ in association with disease states, including a variety of gastrointestinal (eg, inflammatory bowel disease [IBD], colorectal cancer, fatty liver disease), metabolic (eg, obesity, diabetes), immune (eg, asthma, arthritis), and behavioral (eg, autism) conditions.

Different Microbiomes in Health and Disease

The relationship between the disease state and the microbiome is complex. To better understand this relationship, the microbiomes of children with IBD in remission and those with active IBD flare were compared.1 Greater microbial dysbiosis (imbalance), which was evaluated by calculating the microbial dysbiosis index (a ratio of remission and flare microbiomes), was associated with worse IBD severity and reduced bacteria species diversity (Figure 1). However, microbial dysbiosis alone could not identify individuals with IBD or with different IBD types.1 This reflects the complex nature of IBD and suggests that, for some individuals, active disease may be driven by genetic or environmental factors (regardless of a moderately healthy microbiome), while for others active disease is driven strongly by microbial dysbiosis.

Understanding the Disease Threshold: Host Genetics, Bacteria, and Metabolites

The gut microbiome has been shown to interact with host genes that control IBD-related processes (eg, immune regulation, bacterial control, and epithelial integrity) and cellular stress, as well as with the host metabolome.2 Although influential genes have been categorized by different functional impact on disease, it is important to remember that there is a high level of genetic heterogeneity in the population, with each person harboring a unique combination of only a few disease-associated gene loci. In addition to their effects on IBD-related processes, host genes have been shown to have an effect on the microbiome composition; thus, an individual’s genes may drive the development of microbial dysbiosis.3 The microbiome can also affect the metabolome, and vice versa, creating a complex interplay between the effects of disease-associated genes, the composition and diversity of gut bacteria, and metabolites.
IBD microbiome ecosystems appear to vary from healthy basal ecosystems to post-disease ecosystems, with a threshold that determines the presence of symptomatic disease. Pre-disease (basal) variant ecosystems are related to microbial composition and metabolite features, as well as patient habits, diet, and genetics. In the post-disease state, the microbial ecosystem is further influenced by disease processes, such as post-inflammation selection, neutral bystanders, and augmenters of tissue damage. A variety of intervention stressors, such as antibiotic use, diet, and resolution of inflammation, can also affect the gut microbiome, potentially pushing the disease state back below the threshold into a non-disease state. Interestingly, patients’ microbiota changed in different ways depending on the intervention(s) received and likely also depending upon their natural pre-disease microbiome composition. Because of these various factors, the basal and dysbiotic ecosystems are likely represented not by a single state, but instead by a lot of variant states that can be acquired over time (Figure 2).

Microbial Metabolites as a Measure of Disease

In recent years, investigations have sought to understand how the microbiome composition affects host disease pathways and thresholds, and microbial metabolites have been highlighted as a potentially important measure of disease. Several different microbiome metabolomics pathways are elevated in individuals with IBD (Figure 3).
A pediatric family-based cohort study evaluated the microbial and metabolic features of IBD. Categorization into patient subsets by either microbial compositions or shared fecal metabolomics signatures showed high correlation between an IBD-associated metabotype (characterized by increased bile acids, taurine, and tryptophan) and an IBD-associated microbial state. Individuals identified as having metabotype 1 were mostly healthy (no IBD); in contrast, metabotype 2 included a mixture of patients with IBD and healthy first-degree relatives, which may point to a pre-disease susceptibility or the presence of subclinical dysbiosis (Figure 4). It is suggested that evaluation of microbiome metabolites may do a better job of categorizing disease than characterization of the microbiome itself.

Ongoing research is aimed at identifying and validating candidate microbial metabolites that are associated with specific IBD functional and mechanistic processes. A metabolite functional screen was used to identify those metabolites that affect a host biologic property/pathway that is known to affect the IBD disease state, such as CD4+ T cell differentiation, monocyte differentiation, and epithelial organoid differentiation.

Although people are very heterogeneous with regard to their intestinal microbiome and associated metabolites, making diagnosis on the basis of microbiome metabolites difficult, it is hoped that some disease-associated products that may push an individual toward or away from the overall disease threshold will be identified. Monitoring metabolite levels over time could provide important information related to changes in
an individual's disease state. Metabolite research may also have therapeutic implications, with the possibility of both organism-targeted treatments (eg, elimination/modified diets or probiotics to change ecosystem composition and function) and metabolite-targeted treatments (eg, restoration of critical metabolites, or enzymatically blocking production of undesired metabolites) aimed at modifying the microbiome towards a more favourable profile.

In summary, microbiota associated with IBD have now been defined, and there is evidence for the role of causal and pre-disease microbiota. Relevant metabolites of IBD-associated microbiota are also now emerging, with biomarker and therapeutic implications. Although focused on IBD, this work has implications for a variety of other gastrointestinal/microbial diseases, including necrotizing enterocolitis and sepsis, which are frequently observed in preterm infants.

References


Creating a Virus-like Antibiotic

Development of a virus-like antibiotic was based on 3 basic principles: 1) the peptide motif should self-assemble into nanoscale virus-like capsids, 2) the virus shells must have appreciable antimicrobial activity, and 3) the virus-like capsids must actively transfer their genes into the human host cell without causing cytotoxic or hemolytic effects.

In nature, polypeptide chains assemble in water into 3D shapes that function at different levels of biological hierarchy. Elementary secondary and super-secondary protein structural motifs can be designed artificially, but more complex levels (ie, quaternary structures) largely remain inaccessible for design. However, if you have just the right secondary structure design, you can create the right “tool kit” that can be used to direct protein assembly at different length scales. For example, viruses vary greatly in their size and complexity, but often their genes encode just a few proteins. The reason for this is that viruses are highly symmetrical structures that self-assemble from multiple copies of the same building blocks, protein subunits (Figure 1). With the right design, small peptide fragments can be sourced from naturally occurring proteins and adapted for the construction of building blocks that can be used for the assembly of synthetic virus-like particles.

Figure 1. Small synthetic virions.
If such peptides possess a moderate antimicrobial activity, the assembled viruses become antimicrobial. Antimicrobial peptides act by binding to and disrupting the bacterial membrane.\(^4\) Nanoscale imaging (topographic and chemical) allows monitoring of the assembly of antimicrobial peptides into transmembrane pores then expand indefinitely into much larger pores through continued recruitment of the peptide monomers (Figure 2).\(^5\) However, if the peptides are pre-assembled into a virus, this virus converts into a pore immediately upon landing on bacterial membranes.

Artificial peptide motifs can even be designed to assemble into uniform and very small (12 nm) virus-like particles.\(^3\) These viruses efficiently infect (but do not harm) human cells and successfully deliver their genetic load (RNA or DNA) to promote or silence a target intracellular process.

**Antimicrobial Activity of Lactoferrin in Human Breast Milk**

As described above, peptide motifs can be adapted from native proteins. Antimicrobial motifs can be found in biologic fluids. For instance, in addition to providing all of the energy and nutrients that infants need for the first few months of life, breast milk protects against infectious diseases. Several components present in human milk contribute to its broad spectrum bacteriostatic activity. Among these is lactoferrin, which is abundant in breast milk.

Upon evaluating the structure and sequence of lactoferrin, it was determined that much of the bacteriostatic activity of this protein is attributed to a highly conserved, tiny fragment (less than a nanometer across) made up of 6 amino acids.\(^2\) In its free, isolated form, the lactoferrin fragment is negligibly antimicrobial. However, when multiple copies of this lactoferrin fragment are assembled at the same time and at the same point on a bacterial membrane, together they can quickly destroy bacteria by poking holes in the bacterial membrane. Similar innate bacteriostatic activity is seen in proteins and peptides from a variety of different organisms with innate host defenses, suggesting a conserved mechanism of action.

**Novel Lactoferrin-based Antibiotic**

To create a novel lactoferrin-based antibiotic using the design principles of the virus architecture, the small, bioactive lactoferrin peptide fragment was triplicated into a triskelion structure to become a self-complementary, self-assembling subunit. This building block then self-assembles into virus-like capsids (Figure 3).

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**Figure 2. Antimicrobial peptide pore development and expansion.**\(^5\)
Because their shells are made of the lactoferrin peptide, the virus-like capsids can selectively bind to the bacteria membrane and disrupt it through the development of pores; however, they do not create pores in human cell membranes (Figure 4). Instead, the virus-like capsids infect human host cells similarly to viruses and then replicate to produce more antimicrobial virus-like capsids. Thus, these artificial lactoferrin virus-like capsids hit a pathogen with a “double-whammy”: first, they attack and destroy the bacteria from the outside, leaving no memory of what just happened, and, second, they permit the drug developer to make use of capsid properties that can operate between and within different cells, including the specific targeting of intracellular pathogens.

References
Caveats Relating to the Evolution of Human Milk

There is increasing recognition that human milk has extraordinarily diverse beneficial effects on infant growth, development, and numerous health outcomes. Yet, there are 2 important caveats to the a priori evolutionary argument for feeding breast milk to humans, especially those born at very low gestation, in whom the use of breast milk needed to be treated as a research question.

Evolutionary Discordance and Human Milk

As a biological secretion, breast milk has evolved over 200 million years. However, in recent times, humans have been subjected to rapid environmental change, resulting in a mismatch between their ancient genes and their modern environment—notably their diet—known as “evolutionary discordance.” The phenotypic manifestation of evolutionary discordance is disease, and this applies to the health and development of modern infants in a number of ways.

For example, the diet of hunters and gatherers had a ratio of n6:n3 fatty acids that approximated 1:1; in contrast, this ratio can be 16:1 in diets common to modern Western civilizations. This great change in modern diet has affected human breast milk in a number of ways. For instance, the lower amounts of n3 fatty acids in modern breast milk could, given their links to ubiquitous eicosanoids, have broad implications for the health and development of the breastfed infants—a field currently under investigation.

Evolution of Human Milk in the Context of Preterm Infants

A major use of human breast milk is for feeding vulnerable premature babies; however, breast milk originally evolved to meet the needs of full-term infants. It is impossible to conceive that human milk has evolved for the needs of preterm, extremely low birth weight infants since they have only survived at a substantial rate in the last 40 years—a miniscule period in the human evolutionary time line.
Clinical Evidence in Support of a Human Milk Diet

Nevertheless, preterm infants have provided an excellent model for the conduct of relevant clinical studies, including randomized trials, which would be nearly impossible to conduct in full-term infants, to evaluate the impact of early human milk feeding on neonatal and later outcomes. A collection of 29 randomized trials from the United Kingdom and further trials and studies from the United States have allowed comparison of infants who have had an exclusively human milk diet with those who have received cow’s milk-based products solely or in combination with human milk, with some study cohorts now followed into adult life.

Despite the potential caveats for breast milk feeding in humans, cited above, there is increasing clinical evidence supporting the diverse effects of an exclusively human milk diet for infants, not only in terms of protecting against major morbidity in the newborn period (eg, decreased rates of necrotizing enterocolitis, sepsis, and death), but also favorable influences on brain structure and size, cognitive function, cardiovascular risk, the structure and function of the heart, pulmonary development, eye health, bone mass, and atopic disease; some of these outcomes studies have up to 28 years of follow-up (Table 1). Further, by reducing the risk of neonatal morbidities associated with long-term adverse consequences, human milk may have an indirect impact on additional morbidities, such as cerebral palsy. The effect size of a number of these outcomes is unexpectedly large.

Human breast milk contains a variety of non-nutritive constituents, including stem cells that might colonize the infant, immune and antimicrobial factors, hormones, growth factors, cytokines, milk fat globule membranes, exosomes, microRNAs, enzymes, oligosaccharides, and bacteria. When breast milk biology is considered in its entirety, it is clear that the pervasive outcome effects that have been discovered are plausible in light of the equally pervasive predicted impact of so many biofactors in breast milk on the biology of the infant. Indeed, it would seem from the emerging data that preterm infants who do not receive an exclusively human milk diet or who receive cow’s milk may suffer extensive and multiple long-term effects on development and morbidity. It is beginning to appear that these changes reflect a quite general biologic down grading of the organism, with major implications for infants, as well as for clinical practice in both pediatrics and adult medicine.

Table 1. Examples of evidence supporting biologic and health effects of human milk in preterm infants.

<table>
<thead>
<tr>
<th>Favorable biologic impacts</th>
<th>References</th>
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Evolution of the Nutritional Components in Milk

Olav T. Oftedal, PhD
Emeritus Scientist of Nutritional Ecology, Smithsonian Environmental Research Center
Edgewater, Maryland, USA

Lactation is a defining and uniquely mammalian trait, providing the massive transfer of nutrients and bioactive components to young via milk and permitting offspring immaturity at birth. To achieve this transfer, the mother's diet is transformed, stored, balanced, and simplified. Lactation is complex and species-specific, and represents a profound evolutionary achievement that involved the long co-evolution of both milk production and offspring development. We can look to past modifications of developmental signalling pathways and morphogenesis (evo-devo) and the molecular evolution of specific milk constituents.

The Evolution of Lactation

Mammary glands—which appear to have derived from apocrine-like glands—initially secreted onto the skin surface and likely provided moisture, protective constituents, and elemental nutrients to eggs and/or hatchlings long before mammals evolved (Figure 1). Synapsids, the ancestors of mammals, laid parchment-shelled eggs that permitted the uptake of exogenous moisture and simple nutrients. However, very little is known about apocrine gene expression, secretory products, or development.

Several key steps were required to transform this initial secretion into a nutrient-dense fluid: 1) conversion of an antimicrobial constituent (c-lysozyme) into a regulator of sugar synthesis (α-lactalbumin), giving rise to lactose and a large variety of lactose-based oligosaccharides; 2) transformation of mineralization protein(s) into large Ca- and P-transporting casein micelles; and 3) incorporation of constituents involved in cellular immunity into the fat globule membrane, so that fat secretion can be greatly upregulated without damage to secretory cells. Nutrient-dense

Figure 1. Mammary evolution.1
milk evolved prior to the Triassic miniaturization of mammalian ancestors more than 210 million years ago. The ancestral mammalian pattern of hatching from an egg followed by a prolonged developmental period dependent on milk persists in living monotremes, such as the platypus and echidnas.\textsuperscript{2} The echidna secretes milk onto its skin (no nipples), which is believed to be the ancestral form of lactation, before the development of placental mammals.

**Species-specific Lactation**

The process of mammary development varies greatly among species, including evidence of the role of triads of mammary hair follicle–sebaceous units (MPSUs) in mammary morphogenesis (Figure 2).\textsuperscript{1} These MPSUs likely derived from apo-pilo-sebaceous units (APSUs; Figure 1).

Mammals that give birth to highly altricial (undeveloped) offspring typically exhibit great changes in milk constituents, including oligosaccharides, over the course of lactation, presumably to accommodate changes in functional development of the offspring. Eutherian milks are thus extremely diverse in composition, whether in terms of water, fat, protein, sugars, or energy.

Figure 2. Mammary gland development.\textsuperscript{1}

**Human Milk Is Uniquely Evolved for Human Infants**

Human babies are born with a low functional ability and slow development to independence, but human milk is adapted to meet the needs of these infants. The cost of human lactation is low compared with other mammals if you look at the energy cost per day, but not when you look at it as a whole process. Upright posture required a reconfigured pelvis and limited birth canal size, which resulted in immature infants at birth. Human milk has a high water/low energy content associated with frequent nursing, which facilitates social bonding. Sugars and other nutrients in milk help to support the development and metabolism of a large brain. Protein corresponds to a very low proportion of the milk energy (6%; compared with 13% in old-world monkeys and 19% in new-world monkeys), which relates to the slow rate of infant growth. The oligosaccharide diversity of human milk is greater than that of any other mammal, facilitating the growth of beneficial gut microbes, and the predominance of type I oligosaccharides is unique to human milk.\textsuperscript{5} Together with antimicrobial constituents, the milk oligosaccharides help to mitigate the dangers of pathogen exposure that result from increased sociality compared with other primates.

Human milk has many characteristics in common with the milk of other primates, but in many ways is distinctive. Although milk production is highly species-specific, the relationship between developmental maturity and the provision/utilization of nutrients is still not well understood across species.

**References**


Clinical Aspects of Human Milk

Mechanisms Affecting the Gut of Preterm Infants in Enteral Feeding Trials (MAGPIE): A Clinician’s Perspective on the Use of Omic Technology to Better Understand Mechanistic Actions in Nutrition Studies

Nicholas D. Embleton, MD, BSc (hons), MBBS (hons), FRCPCH
Consultant Neonatal Pediatrician, Newcastle Hospitals NHS Foundation Trust; and
Honorary Reader in Neonatal Medicine, Newcastle University
Newcastle upon Tyne, UK

Preterm birth affects >15 million infants worldwide every year. The survival of preterm infants has steadily improved over the past 20 years. However, necrotizing enterocolitis (NEC) and sepsis remain common reasons for morbidity, and the proportions of deaths due to these comorbidities appears to be rising. Recently, there has been increasing recognition of the importance of better nutrition in the prevention of NEC and sepsis, particularly with regard to the use of human milk, and this is expected to become more important as the survival of very preterm infants continues to improve overall.

There is accumulating data suggesting that NEC results from “abnormal” interactions between microbes, the immune system, and nutrition/feeding. However, our understanding of the mechanisms underlying NEC remains incomplete: the usefulness of much of the basic scientific research is limited because animal models of NEC may not adequately reflect clinical reality, and clinical research into the prevention of NEC is complicated by a variety of factors. NEC is really a pathological term that has been used for a clinical syndrome and therefore represents one end of a continuum of gut health rather than a single disease. Causation varies and can be unpredictable, the early signs of NEC can be subtle, and definitions and radiological interpretations are not always consistent. Variation is also seen in the interventions employed, for example, the length of time feeds are discontinued or thresholds for performing a laparotomy. Additionally, clinical studies are limited by the ethical and practical challenges associated with research on small preterm infants.

Omic Approach to Clinical Research

Although large, high-quality, pragmatic trials are essential for exploring key areas of clinical uncertainty and changing clinical practice, they do not provide mechanistic insights. To address the need to better understand the mechanisms underlying NEC and infection in preterm infants, researchers at Newcastle upon Tyne Hospitals and Newcastle University have employed a multi-omics approach to explore the interactions between gut microbes and nutrition (Figure 1). Microbes produce key metabolites and nutrients (eg, short-chain fatty acids, amino acids, bile acids, phenols, and choline) that can impact the host and also affect the environment for other microbes. For example, the production of short-chain fatty acids by key bacterial taxa may impact the promotion of T regulatory cells, cytokines, antimicrobial peptides, and mucous production, as well as the gut-brain axis. Loss of lactobacilli and bacteroides was associated with altered levels of urinary 4-cresol metabolites in inflammatory bowel disease. At the same time, host dietary nutrients (eg, amino acids, urea, human milk oligosaccharides, lactoferrin, fatty acids, and carbohydrates) influence the gut bacterial community, creating a multi-directional, interactive process.

Figure 1. Model for studying feeding exposures and gut health/disease.
Observational Studies on NEC and Sepsis

The Supporting Enhanced Research in Vulnerable Infants (SERVIS) observational study, which was initiated in 2010, has collected samples from >500 preterm infants (<32 weeks gestation) to facilitate analyses into the interactions between the preterm infant gut, nutrition, and infection. All samples were collected by non-invasive methods, such as the collection of blood leftover after routine tests and stool, urine, breast milk (left in syringes or tubing after feeds are finished), and nasal and oral secretions that would normally be disposed of.

Work from our group and others have shown clear differences in the gut microbiota of neonatal intensive care unit (NICU) infants who developed NEC or sepsis versus those who did not. Interestingly, differences tended to reflect changes in the overall structure of the bacterial community rather than changes in specific taxa. The presence of enterobacter and staphylococci were associated with the development of NEC and late-onset sepsis, and this appears to be accompanied with very low rates of colonization with bifidobacteria or lactobacilli in these infants.6 An analysis of stool from twins at risk of NEC and sepsis found that, although the infants had differing exposures, twins had similar stool microbiota, suggesting that a shared environment (eg, breast milk) and/or genetic factors may have a significant impact on early microbial diversity.7

An analysis of microbiomic patterns at discharge found that, while NICU infants had a limited microbiome taxa early in their stay in the NICU, their microbiome became more diverse once the infants were a little older and discharged from the NICU (Figure 2).8 Ultimately, preterm infants were shown to develop comparable gut microbiome complexity compared with healthy full-term infants, despite limitations in early environmental exposures, high antibiotic use, and the frequent presence of serious disease.

Proteomic and metabolomics analyses detected no single protein or metabolite that was consistently different between healthy infants and those with NEC or late-onset sepsis, although several proteins were observed as having an association with disease (Figure 3).9 The lack of clear biomarker proteins and metabolites may relate to different underlying pathophysiology among the infants, further highlighting the complexities of NEC/sepsis diagnosis and treatment.

Figure 2. Increasing bacterial diversity over time.8

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Figure 3. Proteomic and metabolomic analyses.9
The MAGPIE Study

The Mechanisms Affecting the Gut of Preterm Infants in Enteral feeding study (MAGPIE) is being conducted to explore mechanistic differences between trial intervention arms and dynamic changes in the period preceding the onset of NEC or late-onset sepsis. The study will include a subset of approximately 480 very low birth weight (VLBW) infants from 10 large NICUs who are enrolled in 2 large ongoing, randomized, controlled trials, each with ≥2,200 very preterm (<32 weeks gestation) and/or VLBW (<1,500 g) infants. The Speed of Increases in Milk Feeds Trial [SIFT; www.npeu.ox.ac.uk/sift] compared the effect of 2 speeds of daily incremental increases in milk feeding (18 versus 30 mL/kg/day) on short- and long-term outcomes in infants. The Enteral Lactoferrin in Neonates [ELFIN; www.npeu.ox.ac.uk/elfin] trial is evaluating the impact of supplemental lactoferrin (bovine), which is known to have direct antimicrobial and epithelial effects and to modify the host immune response, on the number of serious infections. These studies are the result of a collaboration of the National Perinatal Epidemiology Unit (NPEU), along with members of the UK Neonatal Nutrition Network, and are conducted in >30 tertiary NICUs and 80 “step-down” units in the United Kingdom and Ireland.

In MAGPIE, a daily stool and urine sample is collected from enrollment (Day 0-2) until hospital discharge, and these samples will be analyzed using microbiomic (16s) and metabolomic (GCMS) techniques. Daily stool samples for future omic profiling are being bio-banked; small “waste” samples from catheters and feeding syringes of breast milk, as well as leftover blood samples, are additionally been collected. Formalin-fixed, paraffin-embedded gut tissue samples from hospital pathology archives are also undergoing transcriptomic profiling to explore immune gene expression in NEC and to identify candidate cell surface biomarkers using immunohistochemistry.

It is hoped that these omic analyses will form a program of ongoing research that helps to improve infant care and survival by identifying disease-specific biomarkers, in the form of bacteria, protein, metabolite, and/or gene profiles, that may provide earlier and more specific diagnosis of NEC and sepsis.

References

Upon birth, the extremely low birth weight (ELBW; <1,000 g) infant experiences an abrupt cessation of amniotic fluid exposure, and the infant’s oropharynx is no longer bathed with protective (immune and trophic) biofactors, which stimulate the immune system and promote intestinal maturation. Many biofactors are also contained in breast milk, particularly in colostrum expressed by mothers of ELBW infants. Unfortunately, clinical instability precludes enteral feeding for very low birth weight (VLBW) and ELBW infants during the first days of life, and post-birth fasting leads to intestinal atrophy and abnormal intestinal microbiota, which contribute to increased risk for feeding intolerance and infections, such as late-onset sepsis and necrotizing enterocolitis (NEC). Once started, enteral feeds are administered via a nasogastric tube, bypassing the infant’s oropharynx and delaying oropharyngeal exposure to protective milk biofactors by up to 10 weeks for ELBW infants. OroPharyngeal Therapy with Mother’s Own Milk (OPT-MOM) represents a feasible and promising alternative for these vulnerable infants.

Biologic Rationale for OPT-MOM

OPT-MOM, which involves placing drops of mother’s colostrum/milk directly onto the infant’s oral mucosa, is a novel concept that serves as a natural substitute for amniotic exposure until oral feeds can be safely introduced in ELBW infants. It was proposed that OPT-MOM can provide sustained and prolonged oropharyngeal exposure to protective milk biofactors, which are absorbed by mucus membranes and/or interact with immune cells within the oropharyngeal-associated lymphoid tissues (OFALT). The concept of OPT-MOM is derived from 3 areas of published research: 1) evidence supporting the immunologic effects of oropharyngeal administration of cytokines; 2) research involving the use of bovine colostrum as a biologic therapeutic for sepsis and inflammatory disorders, as well as ulcers, lesions, and infections of the oral cavity; and 3) biochemical and immunologic studies of the protective biofactors in human milk and amniotic fluid, which are similar except for the much higher concentrations of many biofactors in human milk.

It is thought that OPT-MOM will result in beneficial immune responses that are separate and distinct from enteral exposure via nasogastric feeds. OPT-MOM may potentially enhance immune function via several distinct mechanisms, including immunostimulatory effects of cytokine interaction with immune cells within the OFALT, the passive mucosal absorption of protective immune and trophic biofactors (eg, TGF-β and lactoferrin), barrier protection against pathogens in the oropharynx (eg, oligosaccharides, soluble IgA, and lactoferrin), local and systemic effects of oligosaccharides, and the protective effects of antioxidants. As such, OPT-MOM can serve as a potential immunomodulatory adjunct therapy for ELBW infants to prevent mortality and infectious morbidities, including NEC and late-onset sepsis.

Clinical Evidence for OPT-MOM

Two pilot studies demonstrated the feasibility and tolerability of buccal swabbing (N = 56) or oropharyngeal administration via syringe (N = 5) of mother’s own colostrum in VLBW and ELBW infants, respectively. At the time, the staff and parents were nervous about dropping colostrum into the mouths of these tiny babies because it had never been done before. Notably, in the second study, all of the infants began to suck on their breathing tube during administration of the colostrum drops.

Retrospective studies in VLBW and ELBW infants reported that oropharyngeal administration of colostrum was associated with an increased rate of breastfeeding (by 9% in ELBW infants), earlier time to reach full enteral feeding, improved growth, decreased incidences of NEC (by 22%-27% in ELBW infants) and late-onset sepsis (by 22% in VLBW infants), and a reduction in positive tracheal aspirates and blood cultures, although results were not consistent among all studies. Of note, in one of these studies, oropharyngeal administration of colostrum was included as part of a standardized feeding protocol.
A small (N = 16) follow-up prospective study by Dr. Rodriguez’s group randomized ELBW infants to either mother’s own colostrum or sterile water (placebo), administered oropharyngeally in a volume of 0.2 mL every 2 hours for 48 consecutive hours. Half of the colostrum (or placebo) was directed to each side of the infant’s mouth, with the tip of the syringe placed along the right/left buccal mucosal tissue and directed posteriorly towards the oropharynx. The most compelling finding was that treated infants reached full enteral feedings (150 mL/kg/day) 10 days earlier compared with the control group (Figure 1). Results also suggested possible immunostimulatory effects, with a clinically relevant large effect size (1.30) in urinary lactoferrin for colostrum-treated infants.

**Figure 1. Time to enteral feeds for ELBW infants.**

Three other small randomized studies have also recently evaluated outcomes following oropharyngeal administration of 0.2 mL colostrum or sterile water given by syringe every 2 to 6 hours to VLBW or ELBW infants. Lee and colleagues reported a significantly lower incidence of clinical sepsis with colostrum (50%) versus sterile water (92%; P = 0.003), as well as improvement in the levels of proinflammatory and immune-protective factors after 1 to 2 weeks, including urinary levels of secretory IgA, lactoferrin, and IL-1β, and salivary levels of TGF-β1 and IL-8. Sohn and colleagues found a significant reduction in the relative abundance of staphylococci at 96 hours after provision of oropharyngeal colostrum (17% in colostrum-treated infants vs 73% in the control group; P = 0.01). In contrast, a study by Romano-Keeler and colleagues (colostrum given only every 6 hours) found no significant differences in salivary immunopeptides or microbial diversity, although infants who received colostrum did have a 16-day reduction in the median length of hospital stay compared with control infants (P = 0.04). Despite the published evidence in favor of OPT-MOM, safety and efficacy have not yet been established in a well-designed, adequately powered, randomized controlled trial. A large prospective, randomized, double-blind, placebo-controlled trial funded by the Gerber Foundation was recently initiated by Dr. Rodriguez’s group and is currently enrolling ELBW infants (<1,250 g) to compare outcomes with OPT-MOM versus placebo (ie, incidences of NEC and late-onset sepsis) and to identify the biomechanisms responsible for the observed beneficial effects of OPT-MOM, including enhancement of gastrointestinal (fecal) microbiota of the infants, improvement in antioxidant defense maturation or reduction of pre-oxidant status, and maturation of immunostimulatory effects and reduction of pro-inflammatory status. The study is expected to enroll a total of 622 ELBW infants by November 2018; the current enrollment is 127 infants, with 20,065 treatments given. Thus far, the infants have received >95% of planned treatments, demonstrating strict adherence to the rigorous research protocol.
Figure 2. Study design of OPT-MOM randomized controlled trial.38

References
Despite significant improvement in the care of preterm infants, necrotizing enterocolitis (NEC) remains a common reason for morbidity and mortality, particularly in very low birth weight infants. NEC results in damage to the intestinal tract of the infants, ranging from local mucosal injury to full necrosis and/or perforation of the intestinal wall. Although some NEC is successfully treated with administration of antibiotics and changes in nutritional administration in most infants, more severe cases require surgical removal of damaged sections of the intestine.

Preservation of Bowel Length and Function

NEC is the most common cause for short gut syndrome in the pediatric population. Bowel resection due to NEC and other causes of intestinal failure can have a profound impact on survival for years after resection. In adults, survival probability remains high for individuals with 100 to 150 cm of bowel after resection, but is significantly lower at 10 years after resection for those with 50 to 99 cm (<60%) and <50 cm (<40%). To maximize the length of the remaining bowel after removal of damaged sections, surgeons may vertically divide a section of healthy bowel, create 2 tubes from it, and then reattach the 2 tubes to create a thinner section of bowel that is twice the original length. Alternately, healthy bowel may be cut to create a sort of zig zag, thereby increasing its length and absorption ability. For some individuals, intestinal transplant may be the best option to preserve adequate bowel length; however, these patients frequently also have liver failure and require a liver transplant in addition to an intestinal transplant. Transplant increases the risk of graft versus host disease and rejection. Novel research is evaluating the use of tissue-engineered intestine using the implantation of intestine-derived organelle units or modified pluripotent stem cells.

Intestinal Adaptation After Bowel Resection

The gastrointestinal tract of micro-premature infants who aren’t yet being fed becomes trophic; when the infants begin to receive enteral feeds, their intestine starts to grow and flourish. A similar effect happens after short bowel resection, as the remnant bowel compensates for the lost tissue through a process termed “adaptation.” Adaptation is characterized by the development of elongated villi and deeper crypts, which increase the mucosal surface area of the intestine and, thus, its capacity for absorption and digestion per unit length of gut tissue. The amount of adaptive change tends to correlate with the length of intestinal resection (Figure 1), and initial changes in the gut profile can be seen as early as 6 hours after resection.

Intraluminal nutrients, hormones, and growth factors and other gastrointestinal secretions are thought to play a role in intestinal adaptation; however, the mechanisms and mediators of this important response are multiple and remain an active topic of research. A parabiosis experiment published in 1978 demonstrated a role for humoral factors in intestinal adaptation—in 2 rats with a shared circulatory system, bowel resection in one mouse resulted in mucosal-adaptive changes in both the operated and non-operated animals.
Factors Contributing to Intestinal Adaptation

Cells of the intestine are constantly turning over and sloughing into the gut lumen, so homeostasis of the gut mucosa requires careful balance between cell proliferation and apoptosis to prevent intestinal atrophy and neoplasia. Epithelial cells proliferate in the crypts, and the post-mitotic cells differentiate and migrate up into the villi, where they are involved in absorption.

Among the candidate humoral factors that have been proposed to play a role in adaptation is epidermal growth factor (EGF), which is found in high concentrations in breast milk. In a rat model, administration of exogenous EGF enhanced intestinal crypt and villi formation following massive small bowel resection. In contrast, inhibition of the EGF receptor (EGFR) was found to inhibit intestinal adaptation. EGF/EGFR signaling mediate several important responses, such as stimulation of enterocyte proliferation, attenuating rates of enterocyte apoptosis, and stimulation of angiogenesis.

Retinoblastoma protein (Rb) has been shown to be important in cell cycle regulation and differentiation of post-mitotic cells of the villi. Intestinal levels of Rb are reduced after short bowel resection, removing its inhibition of cell cycle progression. In mice, disruption of Rb expression induced mucosal hyperplasia in the small intestine (Figure 2), a phenotype that looks much like the adaptation response seen after surgical resection. Surgical bowel resection in mice with disrupted Rb expression results in an even greater adaptation response. This hyperplasia in Rb-deficient mice is mediated, at least in part, by elevated expression of insulin-like growth factor (IGF)-2.

Interestingly, apoptosis in the intestinal crypts also increases during the week following massive bowel resection. The expression of several apoptotic genes has been found to be altered during the adaptive response, and it is the combined effects of many alterations in gene expression that provides overall regulation of apoptosis and balance with the increase in cell proliferation. For example, a study in mice found that the levels of Bax protein increased by almost 60% following resection, while the levels of Bcl-w were halved; these changes corresponded to an increase in enterocyte apoptosis. The greatest changes in Bax expression occur in the intestinal crypt; with increased Bax expression, the cells die before they make it into and up the villus. The complexity of intestinal homeostasis is demonstrated by the regulatory role of EGFR signaling on Bax and Bcl-w regulation.

The growth of the intestinal villi seen during the adaptation response to bowel resection cannot occur without the development of new blood vessels to support increased blood flow. Immediately following short bowel resection, blood flow is decreased, resulting in a hypoxic environment that stimulates a pro-angiogenic signal. Angiogenesis helps to restructure and expand the blood vessels network, optimizing the vessels for the revised intestine and improving blood flow.

Intestinal Adaptation and Metabolic Changes

Laboratory research has shown that mice gain weight following massive intestinal resection, but have a lower rate of metabolism. In the weeks following resection, the mice fully restore their body fat; however, overall body weight remained low due to reduced lean muscle mass. Similar effects have been observed in the clinic, with those patients with the shortest gut tending to have higher body mass indexes resulting from low lean muscle mass and normal to high fat stores. It is hypothesized that these metabolic alterations toward a more obesogenic profile may be due, in part, to changes in the gut microbiome.
Data published during the past few years have helped to provide a more comprehensive understanding of the process of intestinal development and adaptation following massive bowel resection. Adaptation involves the interaction of several complex processes, including increased enterocyte proliferation and apoptosis, angiogenesis/hypoxia, and alterations in the host metabolic response and microbiome.

**Figure 3. Impact of massive intestinal resection on mouse body composition.**

![Graph](image)

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*P <0.008.
Improving Human Milk and Breastfeeding Outcomes for NICU Infants With the Spatz 10-Step Model

Diane L. Spatz, PhD, RN-BC, FAAN
Professor of Perinatal Nursing and Helen M. Shearer Professor of Nutrition, University of Pennsylvania School of Nursing; and Nurse Researcher – Lactation, Children’s Hospital of Philadelphia
Philadelphia, Pennsylvania, USA

Recognition of the importance of breastfeeding and a human milk diet for infants has increased over recent years. In the United States, there has been a focus on implementation of the Baby-Friendly Hospital Initiative (BFHI), which aims to ensure that all mothers have the information, confidence, and skills necessary to successfully initiate and continue breastfeeding their babies. Currently, there are 395 BFHI-designated US hospitals and birthing centers, and these “baby-friendly” facilities now account for 19.4% of births annually, compared with only 2.9% in 2007. However, the BFHI does not effectively address the needs of infants requiring care in a neonatal intensive care unit (NICU).

10 Steps to Promote and Protect Human Milk and Breastfeeding in Vulnerable Infants

The goal of Dr. Spatz’s “10 Steps to Promote and Protect Human Milk and Breastfeeding in Vulnerable Infants” is to close the current gap in care that results in our most vulnerable infants, including preterm infants, who start their life in a NICU. These infants are most in need of human milk, being the least likely to receive human milk at discharge from the NICU. The model also highlights the critical role of nurses with specialized training in lactation support.

STEP 1: Informed decision

Many mothers make the decision not to pump or breastfeed simply because they haven’t been educated on the importance of human milk or haven’t received the necessary support to initiate breastfeeding. Personalized 1:1 prenatal consultation and the provision of educational resources, such as the Power of Pumping DVD will assist families in making an informed decision and understanding what to expect during the NICU stay. The provision of human milk should be seen as an important medical intervention for all infants, with positive impacts related to protection from infection, reduced risk of necrotizing enterocolitis, feed tolerance, improved developmental outcomes, and protection from both short- and long-term medical conditions.

In addition, many women find the ability to breastfeed or pump empowering. For mothers with infants in the NICU, this act is particularly important because they often feel out of control or helpless to care for their infants. When breastfeeding is not possible, the mother’s focus should be on pumping and the provision of human milk to the infant through other methods. Providing milk for their infants allows mothers to participate and remain engaged in the care of their infants.
STEP 2: Initiation and maintenance of milk supply

It is now recommended that pumping be started within 1 hour after birth. In a pilot study of 20 women randomized to initiate milk expression either within 60 minutes or between 1 and 6 hours after delivery, early initiation was associated with statistically significantly greater milk production after both 1 week \((P = 0.05)\) and 3 weeks \((P = 0.01)\) and earlier lactogenesis stage II \((P = 0.03)\). Encouragement from nursing staff and the availability of pumps by all post-delivery bedsides can help to facilitate early pumping. In cases where pumping isn’t initiated within the first few hours, a chart audit can help the staff to better overcome barriers with future mothers.

STEP 3: Human milk management

Hospital management of human milk is essential for a successful breastfeeding/human milk program. In addition to adequate refrigerator storage, the hospital should have systems in place to ensure the correct infant receives the milk, to identify colostrum versus milk, and to track the timing and amount of pumped milk, as well as how much milk the infant is eating. Care should be taken to ensure mothers don’t unexpectedly run out of milk.

STEP 4: Oral care and feeding of human milk

Oral care should be initiated as soon as possible, given every 2 to 3 hours around the clock until the infant is able to transition to feeding by mouth. While oral care doesn’t get the milk into the infant’s stomach, the infant does absorb some of the milk through the buccal mucosa. Additionally, oral care decreases the risk of the infant developing sepsis. As with pumping, oral care is also a process that infants’ parents can actively participate in, helping them to feel empowered.

STEP 5: Skin-to-skin contact

Continuous skin-to-skin contact from birth is not an option for NICU infants, but contact should be integrated as soon as their diagnosis and stability permits. Although parents and nurses are often hesitant with regard to the initiation of skin-to-skin contact for NICU infants due to concerns about possible contamination, even infants who are intubated or have IV lines can and should receive skin-to-skin contact.

STEP 6: Non-nutritive sucking at the breast

The goal is to get infants to the breast for feeding as soon as possible, and non-nutritive sucking allows the infant to practice the motion of sucking even before they are able to breastfeed. Non-nutritive sucking should be initiated soon after birth, or as soon as the infant is extubated (if intubated after birth). In the NICU, non-nutritive sucking may be accomplished via either suckling at a recently pumped breast (to prevent transfer of milk) or a pacifier. Ideally, the empty breast!

STEP 7: Transition to direct breastfeeding

Infants should be transitioned to direct breastfeeding as soon as possible, even if the infant is still in the NICU. Although infants may not receive much milk on the first few breastfeeding attempts, several attempts should be made. Many NICU infants don’t feed on demand or feed with less vacuum suction than healthy full-term infants, so additional education and assistive technology may be necessary to achieve successful breastfeeding.

STEP 8: Measurement of milk transfer

Weighing infants before and after feeding facilitates objective tracking of infants’ milk intake and ensures that each infant is not over or under fed.

STEP 9: Preparation for discharge

Prior to discharge, hospital staff should ensure that mothers are producing ample milk, as milk transfer during breastfeeding occurs more easily when milk is readily available in the breast, and that the mothers have grown comfortable with direct breastfeeding. Although cue-based feeding is difficult to do in the NICU setting, it is essential to facilitate successful breastfeeding. Cue-based feeding will require mothers to stay in the NICU with their infant for prolonged periods during the day or overnight (6-12 hours), as the infant may breastfeed every 1 to 3 hours. Pre- and post-feeding weights allow tracking of the infant’s milk intake.

STEP 10: Appropriate follow-up

For many NICU infants, continued monitoring of milk intake via pre- and post-feeding weights and continued pumping to supplement breastfeeding is necessary after discharge. Most NICU babies are not efficient at
breastfeeding until at least 2 weeks after the mother’s anticipated due date. Therefore, follow-up with mothers of NICU infants after discharge is important, as is providing access to a health care provider who is knowledgeable about breastfeeding NICU infants.

Implementation of the 10-Step Model

Dr. Spatz developed training programs for health care professionals that provide them with the framework and tools to change practice through implementation of the 10-step model and to achieve measurable results that are personalized, convenient, cost-effective, and innovative. This model was implemented at the Children’s Hospital of Philadelphia (CHOP) over 10 years ago; now, 99% of women in the program initiate pumping for their critically ill infants, and over 86% of infants born in the Special Delivery Unit or admitted within 7 days to the unit are discharged on human milk (Figure 1). Among 186 families with infants discharged from the CHOP NICU, the average and median duration of feeding with human milk was 8 weeks.

The 10-step model has since been implemented successfully across the United States and worldwide in countries such as Thailand, India, and China, with exceptional clinical outcomes. At the Tampa General Hospital NICU, a 3-year continuous quality improvement project focused on implementation of the 10-step model resulted in significant improvement in the proportions of mothers expressing milk within 6 hours of delivery and of VLBW infants receiving mother’s milk at discharge (P <0.001). Mothers also indicated improved satisfaction with the level of nurse breastfeeding support (change in mean Press Ganey satisfaction score from 75 to 93.8).7

References

Impact of Maternal Obesity on Lactation: Finding Clinical Relevance With Mouse Models

Paul S. MacLean, PhD
Professor of Medicine and Pathology, University of Colorado School of Medicine
Aurora, Colorado, USA

Over 60% of adults in the United States are overweight or obese, and the excess weight is associated with a number of co-morbidities that impart an immense burden in medical costs and quality of life. It is clear we are passing this problem to the next generation, as obesity rates have doubled in children and quadrupled in adolescents over the last 30 years. Over 60% of US pregnancies in recent years were carried by women who were overweight or obese, which increases the child’s predisposition for childhood obesity.

Obesity and Early Metabolism

Hales and Barker’s “thrifty phenotype hypothesis” suggests that transgenerational transmission of obesity risk begins while the fetus is still in utero, adapting to the perturbed metabolic environment (nutrient restriction or nutrient excess) within the mother’s womb. A mother’s obesity can thus permanently influence systems that affect energy homeostasis, which results in increased susceptibility to obesity and metabolic diseases, including type 2 diabetes. Conceptually, this influence likely extends to the postnatal period in perturbations to the mother’s milk supply (Figure 1). Obese women have been shown to have problems breastfeeding, including delayed initiation, difficulty sustaining neonatal growth, and early cessation/transition to formula supplementation, even though overweight women (and those with poor diets) tend to produce milk with a higher fat content. Maternal obesity is also associated with larger birth weight, increased neonatal adiposity, and accelerated infant growth. However, the unique effects of obesity versus poor diet on perinatal programming of long-term health and disease are not yet well understood.

Effect of Maternal Diet and Obesity on Lactation Energetics

We investigated the differential effects of maternal diet and obesity with mouse models of diet-induced obesity, focusing on how obesity and an obesogenic diet affect lactation and offspring metabolism. C57Bl/6J mice were fed a high-fat diet with limited activity and then separated into mice that became obese and those that stayed lean despite the obesogenic diet. These 2 groups of mice, as well as lean mice fed a low-fat diet, were followed through pregnancy and lactation. Metabolic phenotyping systems (eg, calorimetry chambers) were employed with nutrient tracers to examine energy balance, nutrient trafficking, and metabolism, in dams and their offspring during lactation.
Regardless of the mother’s adiposity, the high-fat diet was associated with a greater volume of milk production by the mother, reflecting accelerated growth and an increased energy demand by the pups; however, pups from obese mothers showed a decline in the rate of weight gain at mid-lactation.² Both lean and obese mothers eating a high-fat diet increased their energy intake and reduced their energy expenditure (making milk from dietary fat rather than de novo). In the lean mice, the excess energy was put into their milk production, whereas in obese mice the mother kept some of the excess energy for herself, resulting in the production of lipid-poor milk (Figure 2) and maternal weight gain.²

The lipid-poor milk produced by obese mothers is accompanied by an increase in the ω-6/ω-3 fatty acid ratio in milk. Using genetically manipulated mouse models, it was observed that specifically modifying the milk ω-6/ω-3 fatty acid ratio attenuates the diet-induced obese phenotype later in life. Recently published clinical results support the notion that a higher milk ω-6/ω-3 fatty acid ratio may promote obesity in offspring, as the fatty acid ratio in the milk at 2 weeks and 4 months after birth positively correlated with the change in infant adiposity over that time (P = 0.038 and P = 0.010, respectively).³

**Impaired Lactation With Obesity**

Lactation may be impaired in obese individuals in part due to poor mammary gland development and function. This may be due in part to prolactin resistance and/or blunted prolactin in response to suckling. Additionally, insulin is recognized as having an important role in sustaining lactation, but recently it has been suggested that insulin signaling may also be involved in mammary gland development during pregnancy. Studies of mammary epithelial cells that were isolated from pregnant mice demonstrated that insulin stimulates lumen formation, mammary cell size, acinar size, acinar casein content, and the formation of lipid droplets.⁴ Mammary-specific disruption of the insulin receptor resulted in reduced alveolar lobule density and mammary epithelial cell differentiation observed mid-pregnancy, as well as changes in the expression of several genes over the time course of pregnancy and lactation. These effects on mammary development translated into significantly reduced growth of pups nursed by mothers with the disrupted insulin receptors (Figure 3).⁴ A separate study found that both obesity and a high-fat diet impaired mammary gland development in pregnant mice, and that obese mice appeared to have developed resistance to insulin-stimulated mammary gland development.
As noted above, mouse studies indicate that, unlike their lean counterparts, obese mothers tend to store part of the excess energy derived from high-fat diets, rather than redirecting it into the production of milk for their pups.\textsuperscript{2} It is a hypertrophic state, with the animals storing the excess energy. The obese mice are also resistant to mobilizing stored fat, as shown by the larger size distribution of mammary gland adipocytes in obese mice (Figure 4).\textsuperscript{5} The level of acetyl-CoA carboxylase-1 (ACC) was found to decrease with a high-fat diet, and even more so in obese mothers fed a high-fat diet, suggesting severe inhibition of ACC activity in the mammary epithelial cells of mice fed a high-fat diet. A high-fat diet was additionally found to switch localization of the inactive phosphorylated form of ACC from the mammary fat pad to the parenchyma. Thus, not only do obese mice fed a high-fat diet have lower levels of ACC, but the ACC present is primarily inactive.\textsuperscript{5}

Thermoregulatory feedback may also be related to a reduced capacity of obese individuals to dissipate the heat generated during lactation, as adipose tissue is a good insulator. The build-up of heat may negatively regulate lactation production.

Taken together, these studies are building a story about how the “Western” type of obesogenic diet and maternal obesity may combine to alter mammary gland development and the composition of milk, with detrimental consequences to the long-term health of offspring.

References

Exclusively Human Milk Diets for Preterm Infants: 3-year Outcomes

Lewis P. Rubin, MD
Chief of Neonatology, El Paso Children’s Hospital; and
Professor of Pediatrics and Biomedical Sciences and Vice Chair of Pediatrics Research, Texas Tech University Health Sciences Center El Paso
El Paso, Texas, USA

Prematurity is associated with a variety of comorbidities related to hyperglycemia, oxidative stress, hypoxia/ischemia, the formation of glycation end products, micro- and macro vascular complications, and inflammation pathways. Researchers have sought to identify optimal nutrition for preterm infants to mitigate complications. Preterm formulas provide consistent delivery of nutrients and support postnatal growth, but are associated with pro-inflammatory effects, alterations in normal gut microbiome development, increased incidence of infections/sepsis, and longer durations of parenteral nutrition. Although human milk is the ideal diet for healthy full-term infants, human milk does not necessarily provide sufficient nutritional support for appropriate third-trimester growth and development. Consequently, ongoing research is needed to evaluate clinical outcomes with human milk in vulnerable preterm infants.

Use of Human Milk in the NICU

An increasing number of clinical trials and observational experience support benefits of a human milk–based diet for preterm infants who are very low birth weight (VLBW; ≤1,500 g) or extremely low birth weight (ELBW; ≤1,000 g), including shorter hospital stays, good growth, and lower rates of necrotizing enterocolitis (NEC). Several organizations now recommend the use of human milk (including donor milk, as necessary), including the American Academy of Pediatrics (AAP) Committee on Nutrition, the American Society for Parenteral and Enteral Nutrition (ASPEN), and the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) Committee on Nutrition, although some of these recommendations are based on weak evidence. A recent survey of level III and IV neonatal intensive care units (NICUs) in the United States found that human milk is increasingly being used, particularly in larger NICUs, with 59% of units now using donor human milk.1 However, large variation exists among different centers in the criteria for the use of donor human milk (from <1,000 to <1,800 g and/or from gestational age <28 to <34 weeks).

Several studies and meta-analyses (although not all) published in the last few years have demonstrated a decrease in the incidence of Bell’s stage ≥2 NEC associated with the use of an exclusively human milk diet (Table 1), despite variation in the populations and protocols. Few prospective, randomized controlled trials have been conducted, and some centers that are already using human milk understandably lack equipoise to randomize some infants to formula.

Human Milk Use in the El Paso Children’s Hospital NICU

The El Paso Children’s Hospital includes a 50-bed, level III regional NICU that admits >1,000 infants, including 50 to 80 VLBW infants (<1,500 g) annually. In 2012, the rate of NEC among preterm infants was high (14.5%) compared with the national average (Vermont Oxford Network data, 3%-12% for VLBW infants). To decrease the incidence of NEC, the hospital administration was presented with a cost/benefit analysis for the adoption of exclusively human milk diets in ELBW infants; projections were derived from estimates based on the expected decline in NEC cases (per then-available literature), the number of “excess” NEC cases, and the costs per infant for medical and surgical NEC.
Table 1. Summary of reported NEC rates (Bell’s stage ≥2).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study type</th>
<th>Other (eg, formula, mixed)</th>
<th>HM only</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sanchez-Tamayo T, et al. <em>Ann Pediatr.</em> 2016. [Ahead of print]</td>
<td>Retrospective</td>
<td>7.7%</td>
<td>0.9%</td>
</tr>
<tr>
<td>Colaizy TT, et al. <em>J Pediatri.</em> 2016;175:100-105.</td>
<td>Monte Carlo simulation</td>
<td>8.2% (mixed); 11.1% (formula)</td>
<td>1.3%</td>
</tr>
<tr>
<td>Spiegler J, et al. <em>J Pediatri.</em> 2016;169:76-80.</td>
<td>Prospective, cohort</td>
<td>2.2%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Alshaikh B, et al. <em>Breastfeed Med.</em> 2015;10:355-361.</td>
<td>Retrospective</td>
<td>OR = 0.32 (95% CI, 0.11-0.93)</td>
<td></td>
</tr>
<tr>
<td>Hermann K, Carrol K. <em>Breastfeed Med.</em> 2014;9:184-190.</td>
<td>Observational</td>
<td>3.4%</td>
<td>1%</td>
</tr>
<tr>
<td>Sullivan S, et al. <em>J Pediatri.</em> 2010;156:562-567.</td>
<td>Randomized, controlled</td>
<td>All NEC: P = 0.02; surgical NEC: P = 0.007</td>
<td></td>
</tr>
<tr>
<td>Meinzen-Derr J, et al. <em>J Perinatol.</em> 2009;29:57-62.</td>
<td>Randomized, controlled</td>
<td>HR = 0.85 (95% CI, 0.60-1.19)</td>
<td></td>
</tr>
</tbody>
</table>

Human milk diets were integrated in a 3-year (fiscal years 2013-2016), stepwise fashion, which facilitates examining staged improvements. During the first year of increased human milk use, the NICU started to provide Prolacta’s Prolact+HMF for infants ≤1,000 g birth weight. Review of several months of data indicated no new NEC cases in ELBW infants, but continued occurrence of NEC in other VLBW infants. In subsequent years, Prolact+HMF and donor human milk diets were adopted for infants ≤1,250 g, and then for all infants ≤1,500 g.

Stepwise adoption of exclusively human milk diets for preterm infants resulted in marked decrease of NEC Bell’s stage ≥2 during the past 2 years among VLBW infants (Figure 1). An analysis of comorbidity rates of infants with exclusively human milk versus “mixed”
(human + bovine-based milk fortified) diets also showed declines in severe intraventricular hemorrhage and mortality (Table 2). In contrast, the frequency of retinopathy of prematurity appeared to increase with an exclusively human milk diet, although more robust data are needed to ensure the data are not due to low patient numbers. Other outcomes seen after integration of exclusively human milk diets included elimination of deaths from NEC; decreases in length of stay, days on total parenteral nutrition, days with an indwelling central venous catheter, and days of antibiotic administration; less long-term morbidity; and lower NICU costs.

Table 2. Outcomes with exclusively human milk versus “mixed” diets in low birth weight infants.

<table>
<thead>
<tr>
<th>Weight group:</th>
<th>500-749 g</th>
<th>750-999 g</th>
<th>1,000-1,250 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet:</td>
<td>Mixed</td>
<td>HM only</td>
<td>Mixed</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>50%</td>
<td>67%</td>
<td>64%</td>
</tr>
<tr>
<td>Severe intraventricular hemorrhage</td>
<td>0</td>
<td>0</td>
<td>9%</td>
</tr>
<tr>
<td>Periventricular leukomalacia</td>
<td>25%</td>
<td>17%</td>
<td>36%</td>
</tr>
<tr>
<td>Severe retinopathy of prematurity</td>
<td>0</td>
<td>33%</td>
<td>9%</td>
</tr>
<tr>
<td>Transfusions, n</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>NEC</td>
<td>40%</td>
<td>0</td>
<td>18%</td>
</tr>
<tr>
<td>Mortality</td>
<td>20%</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Exclusively human milk diets are a practical, nutritional strategy to improve survival and health, and decrease hospital-related morbidities in VLBW and ELBW infants. In human milk, bioactive factors such as long-chain polyunsaturated DHA, choline, and lutein show marked variation among individuals and communities (1-2 orders of magnitude). Further investigations of clinical biomarker profiles, micronutrient intakes, and the impact of human milk components on longer-term outcomes are needed.

Figure 1. NEC (medical/surgical) rates in VLBW infants.

Reference
