Scientific Evidence for Breastfeeding

Alan Lucas

The global drive to promote breastfeeding targeted at all 134 million infants born/year on the planet is one of the most pervasive public health interventions. It is therefore critical that the breastfeeding field is evidence based. Three key scientific pillars of breastfeeding have been: (1) that human milk (HM) is ideally adapted for babies since it is the product of 200 million years of mammalian evolution; (2) that HM composition should be seen as the gold standard for infant nutritional requirements; and (3) that HM has numerous clinical benefits for the infant. All of these 3 contentions can be challenged on the grounds that the underlying evidence and thinking has been significantly flawed. I shall identify these flaws to help pave the way to a more solid basis for modern breastfeeding medicine.

Firstly, the incorrect use of the evolutionary theory for human breastfeeding is dissected, notably the evidence in humans for a mismatch between our rapidly changing environment and our ancient genes (evolutionary discordance). The possible evidence for evolutionary discordance in relation to breastfeeding is broad-based and includes consideration of the risk of vitamin K deficiency bleeding, vitamin D deficiency, iron deficiency, n-3 fatty acid intake, and cardiovascular disease risk. The practical implications of evolutionary discordance for optimal nutritional care of breastfed infants are discussed.

Secondly, I shall show how HM composition has been incorrectly translated into dietary intake in a large body of past flawed work that resulted in misleading data. By the 1960s, there had been 1,500 publications on breast milk composition; yet, it is difficult to obtain representative samples of breast milk because milk fat changes during a feed. The past studies greatly overestimated fat and energy in breast milk. Also, breast milk protein content was often estimated from nitrogen content as employed in the dairy industry. However, because of the high non-protein nitrogen content of human milk, this methodology led to overestimation of breast milk protein content. Unfortunately, these incorrect compositional data were used as a model for the design of infant formulas.
resulting in more rapid growth. Evidence is presented to show that accelerated early growth contributes to the modern epidemic of obesity and cardiovascular disease risk (“postnatal growth acceleration hypothesis” [1]). Better approaches to the determination of breast milk nutrient content (for instance using stable isotopes) have helped generate more appropriate data to underpin infant nutrition requirements, guide design future formulas, and provide understanding of the beneficial effects of breastfeeding in relation to reduced later risk of obesity and cardiovascular disease.

Finally, most studies purporting to show benefits of HM are observational and potentially confounded, so causation cannot be proven. Thus, hard experimental evidence is required. However, in term infants, randomized trials of breast versus formula feeding are ethically difficult and seldom done. Here, I shall use preterm infants as a model, since numerous randomized controlled trials and physiological studies over 40 years have compared exclusive HM feeding versus cow’s milk exposure. Unexpectedly diverse immediate beneficial effects span the field of neonatology, and long-term programmed effects have been shown for cognition, brain structure, risk factors for cardiovascular disease, structural development of the heart and lungs, bone health, and atopy. These data add much weight to the evidence obtained in full-term infants using weaker study designs that HM feeding in early life may fundamentally and permanently change the biology, health, and developmental outcomes of the organism.

With these advances in science and thought, breastfeeding is emerging as a major evidence-based field of medical and public health practice.

Reference

The Biomechanics of Breastfeeding: Bridging the Gap between Engineering-Based Studies and Clinical Practice

Mike Woolridge

Understanding how a baby extracts milk from the breast is the vital cornerstone to practicing sound, effective breastfeeding management in order to optimize milk transfer from mother to baby. In turn, this allows one to maximize the transfer of calorie-rich nutrients, predominantly of breast milk fat.

For several centuries, received wisdom was that babies extract milk from the breast by a combination of baseline suction, compression, and relaxation of the baby’s jaws against the breast, and rhythmical waves of pressure applied to the underside of the breast/nipple held within the baby’s mouth by the tongue [1]. Based on this premise, the core principles of WHO/UNICEF training were established, focusing on optimizing the positioning and attachment of the baby at the breast in order to maximize the effectiveness of milk transfer.

In the past decade, however, this received wisdom has been challenged both by the use of modern ultrasound equipment [2, 3] and engineering-based modeling of breast anatomy (specifically the milk duct system) and the baby’s sucking action [4, 5].

A key novel claim was that the baby can generate localized, added suction with its tongue to enhance milk transfer [2, 6]; this has since been confirmed [7], although the evidence is that this novel mechanism remains secondary to the core process of peristaltic expression by the tongue.

In contrast, engineering-based studies [4, 5] have proven both controversial and contradictory, providing new insights yet posing fresh challenges. To date, however, they have not altered the core underpinnings of best breastfeeding practice and management.

In the field of medicine, it is recognized that the validity of randomized controlled trials should be evaluated by a set of quality control
standards, and the framework of critical appraisal skills is a way of achieving this. No such quality standards or guidelines exist for evaluating engineering-based models of a physiological process. A comparable framework is needed if the validity of engineering-based models is to be effectively assessed. In practice, in order to address the veracity of the conclusions drawn, it is essential to be able to evaluate several of the assumptions made in these models: whether or not they are valid, and whether specific elements are missing from current models which might affect their outcome.

Certain physical assumptions, made during the modeling process, are known to be incorrect, but have been made in order to simplify the modeling process – for example, that the milk duct walls are rigid. Further ways in which the modeling process departs from known physiology include: (i) the view that negative suction pressure is the primary force in these models, without any contribution being made by the progressive peristaltic pressure exerted by the baby’s tongue [8], and (ii) the core assumption that the milk duct system remains patent throughout a feed, thereby ignoring the occlusive impact of the baby’s jaw closure with each suck. The inclusion of any of these natural processes would radically alter the conclusions from modeling, thereby disproving the claim that suction alone can explain milk extraction [4] while giving greater credence to the suggestion that suction alone may not fully explain milk extraction [5].

One feature consistently missing from such analyses is the clinical implications arising from them, and what they add to our understanding in terms of how to help mothers and babies breastfeed more effectively. To this end, a pivotal role played by peristaltic tongue movements, essential to effective breastfeeding, will be identified and elaborated, so providing evidence as to why the core management principles of positioning and attachment are so central to breastfeeding success.

References


Physiological Effects of Feeding Infants and Young Children Formula Supplemented with Milk Fat Globule Membranes

Olle Hernell, Magnus Domellöf, Tove Grip, Bo Lönnerdal, and Niklas Timby

An increasing number of studies have reported different health benefits from oral supplementation with bovine milk fat globule membrane (MFGM) to infants and children (Table 1) [1, 2]. MFGM is a biologically active milk fraction that contains a large proportion of milk phospholipids, sphingomyelins, and gangliosides together with several hundred identified proteins, including mucins, butyrophilin, lactoferrin, and lactadherin (Fig. 1). Formula-fed infants are of special interest with respect to MFGM supplementation since they have a lower intake of MFGM components compared to breast-fed infants because, traditionally, the MFGM fraction is discarded with the milk fat when this is replaced by vegetable oils as the fat source in infant formulas.

Clinical Studies on the Effects of MFGM Concentrates Fed to Infants and Children

In the first double-blind, randomized, controlled trial (DBRCT) in 550 healthy, primarily breast-fed 6- to 11-month-old infants, supplementation with an MFGM-enriched protein fraction reduced diarrheal morbidity [3]. In another DBRCT in 70 infants, supplementation with bovine milk gangliosides, provided as a complex bovine milk lipid fraction from 2–8 until 24 weeks of age, increased hand-eye coordination, performance, and general IQ after adjustment for socioeconomic background variables [4]. A third DBRCT including 253 preschool children aged 2.5–6 years evaluated a daily intake of a formula enriched with 500 mg of phospholipids with the addition of a phospholipid-rich MFGM concentrate for 4 months, and found reduced days with fever and less behavioral problems during the intervention [5]. In an Indian DBRCT, 450 infants between 8
and 24 months of age were randomized to a daily dose of milk powder supplemented with 2 g of a spray-dried ganglioside concentrate or milk powder only for 12 weeks [6]. There was no difference between the groups either in the primary outcome rotavirus diarrhea or in the secondary outcomes, including all-cause diarrhea. However, the authors noted that the incidence of rotavirus diarrhea during the study period was lower than expected, making the study underpowered compared to the intention of the design. In a Swedish DBRCT in 160 formula-fed healthy term infants, supplementation with a protein-rich MFGM fraction from <2 until 6 months of age improved cognitive scoring in Bayley III [7]. Further, a reduced incidence of acute otitis media, a reduced antipyretic use, lower concentrations of serum IgG against pneumococci after vaccination, and a lower prevalence of Moraxella catarrhalis in the oral microbiota suggested

**Table 1.** Double-blind, randomized controlled trials exploring the effects of milk fat globule membrane (MFGM) supplementation to the diet of infants or children

<table>
<thead>
<tr>
<th>Study</th>
<th>Age</th>
<th>Supplementation</th>
<th>Main results for MFGM groups</th>
</tr>
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<tbody>
<tr>
<td>Zavaleta et al. [3]</td>
<td>6–11 months</td>
<td>MFGM (Lacprodan® MFGM-10, Arla Foods Ingredients)</td>
<td>Lower longitudinal prevalence of diarrhea Lower incidence of bloody diarrhea</td>
</tr>
<tr>
<td>Veereman-Wauters et al. [5]</td>
<td>2.5–6 years, for 4 months</td>
<td>MFGM (INPULSE®, Büllinger SA)</td>
<td>Fewer days with fever and lower parental scoring of internal, external, and total behavioral problems</td>
</tr>
<tr>
<td>Poppitt et al. [6]</td>
<td>8–4 months, for 12 weeks</td>
<td>Complex milk lipids (Fonterra Cooperative Ltd)</td>
<td>No difference between groups</td>
</tr>
<tr>
<td>Timby et al. [7–9, 11]</td>
<td>&lt;2 to 6 months</td>
<td>MFGM (Lacprodan® MFGM-10, Arla Foods Ingredients)</td>
<td>Higher cognitive score Lower incidence of otitis media Higher serum cholesterol</td>
</tr>
<tr>
<td>Billeaud et al. [10]</td>
<td>14 days to 4 months</td>
<td>Lipid-rich MFGM fraction (Fonterra Cooperative Group)</td>
<td>Weight gain was noninferior Higher rate of eczema in the protein-rich MFGM group</td>
</tr>
</tbody>
</table>


an infection-protective effect of MFGM supplementation [8, 9]. In a non-inferiority DBRCT in 199 healthy term infants from 14 days to 4 months of age, a formula enriched with lipids and a formula with a protein-rich bovine MFGM fraction yielded a noninferior weight gain with no serious adverse events compared with a standard formula [10].

**Conclusions**

Studies investigating the effect of bovine MFGM-supplemented diets on infants and children have shown promising results regarding both neurodevelopment and defense against infections. However, the scientific base of knowledge for MFGM supplementation to infants and children

**Fig. 1.** Schematic drawing of the release of the milk fat globules and composition of the MFGM. Illustration by Erik Domellöf. Reproduced from Hernell et al. [1] with permission.

Milk fat globule

MFGM

MFGM proteins

Glycerophospholipids

Sphingolipids

Cholesterol

Glycosphingolipids
is still limited. The number of studies published on MFGM provided to infants and children is small, and the interventions are heterogeneous: different MFGM concentrates have been given for different durations at different infant/child ages and with different main outcomes. However, MFGM supplementation seems safe down to the age of the first week of life in term infants, as no serious adverse effects have been reported.

Infant formulas supplemented with bovine MFGM concentrates have already been launched on many markets, but before firm conclusions can be drawn on the likely health benefits of supplementing the diet of infants and children with MFGM, more high quality DBRCTs are needed.

References
Breastfeeding is related to a lower risk of infections and possibly diabetes and overweight in later life, while the situation for allergies is less clear [1], which suggests that breast-milk-specific components may contribute to such benefits. Among them are the nondigestible human milk oligosaccharides (HMOs), the third largest solid breast milk component. HMOs are elongations of the milk sugar lactose by galactose, N-acetylglucosamine, fucose, and sialic acid, which results in structures similar to those on the mucosa. Most HMOs are not present in farmed-animal milks and are different from generic prebiotics such as galacto- and plant-derived fructo-oligosaccharides. Maternal fucosyltransferases FUT2 and FUT3, encoded by the Secretor and Lewis genes, respectively, followed by lactation stage, have the most striking impact on the HMO composition [2]. The presence or absence of functional FUT2 and FUT3 not only affects the abundance of individual fucosyl-HMOs, but also the total HMO concentration in breast milk. The maternal nutritional and health status might influence HMO composition in the breast milk; however, today there are only circumstantial data to this end. Clinical observational studies in breastfed infant-mother dyads associate specific HMOs with infant gut microbiota, morbidity, infectious diarrhea, and allergies. Although observational studies do not establish causality, together with experimental data they suggest possible biological roles for HMOs. In particular, it is believed that they affect the (i) establishment of the early-life microbiota dominated by bifidobacteria, (ii) resistance to pathogens, and (iii) intestinal mucosal barrier and immunity, thereby contributing to immune protection (Fig. 1a) [3].

Clinical intervention trials with infant formula supplemented with 1 HMO (2′fucosyllactose, 2′FL) or 2 HMOs (2′FL with lacto-N-neotetraose) demonstrated that they allow for age-appropriate growth and are well tolerated [4, 5]. A priori defined secondary outcomes suggested that
Fig. 1. Illustration of the different biological functions of HMO (a) and risk for infection-related illnesses and medication use in infants fed a formula supplemented with 2 HMOs (redrawn from Puccio et al. [5]). Illnesses and medication use were reported by parents and verified by a study physician (b). URT, upper respiratory tract; LRT, lower respiratory tract. Odds ratios with 95% confidence intervals are shown based on percent of infants with at least 1 event at 1 year of age (Fisher’s exact test: * p < 0.05, ** p < 0.001) [6].
feeding an infant formula with 2 HMOs relates to fewer reported lower respiratory tract illnesses and reduced requirement for related medication (antibiotics and antipyretics) during the first year of life [5]. In parallel, the early-life microbiota composition and community structure in infants fed the 2-HMO formula shifted towards that of breastfed infants. Formula containing 2 HMOs shifted the global microbiota profile towards that of breastfed infants, characterized by a *Bifidobacterium* dominance and lower abundance of *Escherichia*, for example. Interestingly, infants with a microbiota community structure typical for control-formula-fed infants had a 2 times higher risk to use antibiotics during the first year of life than those with a microbiota community typical for breastfed infants.

Together, clinical observational studies corroborated by preclinical experimental data and clinical intervention trials support a role for specific HMOs in immune protection leading to the reduced use of antibiotics. Further clinical studies, well-designed observational and especially placebo-controlled interventions, are warranted to further substantiate and grow our understanding of the HMO biology and significance for infant nutrition.

**References**

Fatty Acids and Fat-Soluble Vitamins in Breast Milk: Physiological Significance and Factors Affecting Their Concentrations

Ardythe L. Morrow and Adekunle Dawodu

The lipid fraction is the second-most abundant solid constituent of human milk, and the most important source of dietary energy. Major constituents of the lipid fraction are fatty acids and fat-soluble vitamins, which are critical contributors to infant health and development. Fatty acids have a critical role in infant neurodevelopment, cardiovascular health, and immune regulation. The fat-soluble vitamins – A, D, E, and K – are critical for infant immune health, neurodevelopment, vision, and modulation of coagulation, and provide antioxidants to minimize cellular damage. Thus, these components are highly bioactive and contribute to infant health and development.

**Fatty Acids**

The fatty acids of human milk are diverse in length and include saturated, monounsaturated, polyunsaturated, and branched-chain structures. The three most abundant fatty acids of human milk – oleic, palmitic, and linoleic acid – comprise about two-thirds of the fatty acid fraction. While there are core fatty acids common to diverse global populations, fatty-acid composition is otherwise highly variable across populations and between mothers, depending on maternal diet and genetics. Well-documented differences in the fatty-acid profile of human milk across populations include linoleic acid, docosahexaenoic acid (DHA), and other n-3 fatty acids, the trans-fatty acids, and branched-chain fatty acids. DHA and other n-3 fatty acids tend to be higher in fish-eating populations; branched-chain fatty acids tend to be found in higher concentrations among mothers who consume more daily servings of dairy and beef; and trans-fats occur significantly more often in the milk of mothers consuming typical western diets but are very low in the milk of women in traditional societies.
The impact of differences in the fatty-acid profile of human milk on infant health is understudied and an important domain of research. The strongest evidence of impact has been shown in preterm infants. The milk of preterm infants, whether mother’s own milk or donor milk, is typically lacking DHA, and infant body stores are limited. Maternal supplementation with preformed DHA could provide an important strategy for improving maternal and infant health.

Table 1. Fatty acids and fat-soluble nutrients in human milk

<table>
<thead>
<tr>
<th>Fatty acid associated</th>
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<tbody>
<tr>
<td><strong>Docosahexaenoic acid</strong></td>
</tr>
<tr>
<td>Increased with intake of fish and other DHA-rich foods</td>
</tr>
<tr>
<td>Differs by population</td>
</tr>
<tr>
<td>Lower in milk of mothers who deliver preterm</td>
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<tr>
<td><strong>Branched-chain fatty acids</strong></td>
</tr>
<tr>
<td>Dairy and beef consumption associated with higher levels of specific BCFA</td>
</tr>
<tr>
<td>Differs by population</td>
</tr>
<tr>
<td><strong>Trans-fatty acids</strong></td>
</tr>
<tr>
<td>Higher in westernized populations</td>
</tr>
<tr>
<td><strong>n-6:n-3 ratio</strong></td>
</tr>
<tr>
<td>Higher in westernized populations</td>
</tr>
<tr>
<td>Fat-soluble vitamins</td>
</tr>
<tr>
<td><strong>Vitamin A</strong></td>
</tr>
<tr>
<td>Typically adequate levels</td>
</tr>
<tr>
<td>Low in low-resource regions (Africa and Southeast Asia) and mothers with low intake of animal foods</td>
</tr>
<tr>
<td>Lower with premature delivery</td>
</tr>
<tr>
<td><strong>Vitamin D</strong></td>
</tr>
<tr>
<td>Typically below detectable levels</td>
</tr>
<tr>
<td>Infant supplementation is recommended</td>
</tr>
<tr>
<td>Milk levels can be increased with dietary maternal supplementation of 6,400 IU/day</td>
</tr>
<tr>
<td><strong>Vitamin E</strong></td>
</tr>
<tr>
<td>Typically adequate levels</td>
</tr>
<tr>
<td>Lower levels with premature delivery</td>
</tr>
<tr>
<td><strong>Vitamin K</strong></td>
</tr>
<tr>
<td>Typically low levels</td>
</tr>
<tr>
<td>Infant status depends on bacterial synthesis or supplementation/injection</td>
</tr>
<tr>
<td>Levels can be increased with maternal supplementation</td>
</tr>
</tbody>
</table>
Fat-Soluble Vitamins

Fat-soluble vitamins A, D, E, and K are vital for infant nutrition. They perform important health functions and can be stored in the liver and fat tissue until required. While human milk typically has adequate levels of vitamins A and E to meet infant needs, there is variation between populations, and levels can be limited in the circumstance of preterm birth. In human milk, vitamin D and K levels are typically limited. The global public health consensus is to supplement all infants with vitamin D for the prevention and management of nutritional rickets. Recent data indicate, however, that supplementation of sufficiently high doses of vitamin D to lactating women (6,400 IU/day) can safely produce clinically relevant levels of milk vitamin D to satisfy the requirement of her nursing infant. Vitamin K is also low in human milk, and direct vitamin K administration to newborns is recommended practice.

Factors associated with human milk concentration are shown in Table 1.

Commentary

Fatty acids and fat-soluble vitamins are the subject of increased attention in public health nutrition. The health of the infant and the impact of natural variation on development observed between populations and mothers is not known. Nutrient deficiencies in human milk may be managed by supplementation of pregnant or lactating mothers or direct supplementation of infants depending on the nutrient. Dietary supplementation with DHA to pregnant mothers is under study. Preterm infants should be considered a nutritionally needy population worldwide. The milk of mothers who deliver preterm infants may be deficient in n-3 fatty acids and fat-soluble vitamins. Focused attention to the fat-soluble nutrient needs and intake of breastfed infants is warranted.

References

Water-Soluble Vitamins in Human Milk: Factors Affecting Their Concentration and Their Physiological Significance

Lindsay H. Allen and Daniela Hampel

Water-soluble vitamins are essential for the breastfed infant’s health and development, yet they are among the most susceptible to depletion in human milk when maternal status and/or intake is low. B vitamins play essential roles in cell metabolism, including DNA synthesis and regulation, fatty acid and amino acid metabolism, and gluconeogenesis, either as cofactors/coenzymes for or as precursors of these cofactors. Vitamin C serves as an antioxidant involved in tissue repair, immune system support, and interferon production. Inadequate supply of one or more water-soluble vitamins to breastfed infants can result in growth retardation, DNA damage, or metabolic defects, and affect the cardiovascular, muscular, gastrointestinal, and nervous systems. Typical deficiency syndromes in infants are beriberi (B1), ariboflavinosis (B2), pellagra (B3), neural tube defects (folic acid), and megaloblastic anemia, growth retardation, and impaired development (B12)[1].

There are natural changes in the concentrations of water-soluble vitamins in human milk over the course of lactation. While vitamins B1 (thiamine), B3 (niacin), and B5 (pantothenic acid) increase throughout the course of lactation, concentrations of vitamin B6, B12, and C decrease. In contrast, vitamin B2 (riboflavin) remains constant, as does choline after an initial increase during the first months of lactation. Folate has a unique pattern with increasing and decreasing concentrations until stabilization in late lactation.

The concentrations of most of the water-soluble vitamins are influenced by maternal status and/or supplementation. While vitamins B1, B2, B3, B5, B6, B12, choline, and vitamin C in milk are quite strongly correlated with maternal status, folate is not. A low maternal intake of animal source foods causes depletion of B12 in the milk although milk B12 appears to be more dependent on maternal liver stores and accumulation in the liver of the fetus. Low intake of riboflavin will also rapidly reduce
its concentration in milk since humans do not have excessive stores of this vitamin. Maternal supplementation positively affects milk concentrations of vitamins B₁, B₂, B₃, B₆, and B₁₂ (Fig. 1) but has no effect on folate. However, the efficacy of maternal supplementation postpartum is somewhat limited [2]. Other factors affecting concentrations of some water-soluble vitamin concentrations in human milk include parity, preterm delivery, diurnal variation, smoking, medication, and maternal illness.

Existing data on the concentrations of water-soluble vitamins in human milk are very limited. Most studies had few participants, some analytical methods were invalid, the vitamin status or intake of the mother was often unknown, and few studies measured concentrations longitudinally during lactation. As a result, there is substantial variation in the reported concentrations that were used to set the adequate intakes for infants and recommended intakes for lactating women. We have developed more efficient, validated methods that can now measure most of the B vitamins and their vitamers simultaneously in small volumes of milk [3]. These have revealed the large differences in concentrations among population groups around the world and enabled the efficient determination of the effects of multiple micronutrient supplements on milk vitamins. This raises the question of how to define a “low” value and is

**Fig. 1.** Percent changes in the concentrations (means ± SEM) of water-soluble vitamins in human milk after maternal supplementation compared to nonsupplemented women (100% value) at 2, 6, and 24 weeks [2]. * p < 0.05, *** p < 0.001, control vs. supplemented groups.
the goal of our ongoing study to evaluate the concentrations of vitamins (and other nutrients) in milk from well-nourished but unsupplemented women in 4 countries during the first 9 months of lactation. This Mothers, Infants and Lactation Quality (MILQ) study will establish reference values that will improve estimates of the nutrient requirements of infants and lactating women and enable the adequacy of milk nutrient concentrations to be evaluated and compared across populations.

References

Human Milk MicroRNAs/Exosomes: Composition and Biological Effects

Bo Lönnerdal

Human milk provides many benefits to the breastfed infant resulting in significantly better short- and long-term outcomes compared to formula-fed infants. These benefits are likely achieved by a well-balanced supply of nutrients and wide variety of bioactive components in breast milk. These components include long-chain fatty acids (e.g., DHA), complex oligosaccharides, bioactive proteins (e.g. immunoglobulins, lactoferrin, and osteopontin), nucleotides, and lutein. By various mechanisms that have been extensively studied, they protect the infant against infections and stimulate brain development and visual function.

More recently, it was discovered that breast milk also contains exosomes, i.e., microvesicles consisting of microRNAs (miRNAs) with sizes of ~22 nucleotides [1]. Exosomes are small extracellular vesicles about 30–100 nm in size and are produced by a variety of cells, including macrophages, lymphocytes, dendritic cells, epithelial cells, and tumor cells. They are found in physiological fluids such as plasma, urine, and malignant effusions. It is well known that exosomes are important in cell-cell signaling, but their physiological significance in vivo is less known. Early studies suggested promising roles in immunotherapy and cancer therapy, but this is a rapidly advancing field, and many clinical trials are ongoing. It was shown that isolated milk exosomes could affect immune responses of PBMCs and T-regulatory cells [1]. A subsequent study on miRNA expression in breast milk found large numbers of miRNAs (281 of 723 human miRNAs known at that time) and, in particular, high levels of immune-related miRNAs during the first 6 months of lactation [2]. Several breast milk miRNAs have been shown to originate from the mammary gland [3], and many of them are involved in cellular development and immune function.

Exosome-mediated transfer of miRNAs is a novel mechanism of genetic exchange between cells. It is therefore possible that exosomes in milk may survive digestion and deliver miRNAs to intestinal cells, or, if transferred into the blood stream, to cells in other tissues. In vitro
digestion experiments mimicking conditions in the infant gut have shown that exosomes and their miRNA cargo can survive proteolytic digestion [4]. Further, when human intestinal epithelial cells in culture were exposed to exosomes isolated from breast milk that was undigested or subjected to in vitro digestion, it could be shown by confocal microscopy that the cells could take up exosomes from both untreated and in vitro digested breast milk and that they migrated to the nucleus [4]. Therefore, it is possible that breast milk exosomes may affect gene transcription in the small intestine. Research on human adults consuming cow milk in various amounts has shown that major bovine milk miRNAs are found in the circulation postprandially and in a dose-dependent manner, further suggesting that exosomes can resist conditions in the gastrointestinal tract and be delivered to the systematic circulation [5]. Thus, it is possible that milk miRNAs may transfer genetic material to the infant and thereby affect gene transcription and regulation of cellular events in several tissues.

References

Human milk (HM) is the ideal food that ensures optimal growth and development of infants [1]. In addition, HM contains a wide variety of bioactive components, including lipids, oligosaccharides, and proteins. Over the past 30 years, infant formulas have undergone dramatic changes in nutritional composition to more closely mimic that of HM [2]. However, clinical and epidemiological studies show that differences in short- and long-term health outcomes still persist between breastfed and formula-fed infants, including growth patterns, nutritional status, gut microbiota composition, prevalence of infection, and health outcomes [1]. HM contains over 400 proteins that can be broadly classified into 3 categories: caseins, whey proteins, and mucins, which are present in the milk fat globule membrane (MFGM). HM is whey predominant, but the whey/casein ratio of HM changes during the course of lactation, being 90/10 in colostrum and changing to 60/40 in mature HM. The predominant caseins in HM are b and k, whereas bovine milk contains a, b, g, and k caseins. The proteins present in significant quantities in the whey fraction are α-lactalbumin, lactoferrin, IgA, osteopontin (OPN), and lysozyme. The predominant whey protein in bovine milk is b-lactoglobulin, although low concentrations of α-lactalbumin, lactoferrin, and OPN in bovine milk have enabled their isolation and utilization in preclinical and clinical trials. Additionally, bioactive peptides are formed during the digestion of casein and whey, and glycans from glycoproteins are bifidogenic, adding further complexity to the functional properties of HM proteins. These functions include: serving as a source of amino acids; improving the bioavailability of micronutrients, including vitamins, minerals, and trace elements, providing stimulation of intestinal growth and maturation; supporting immunologic defense; shaping the microbiome; and enhancing learning and memory (Fig. 1) [2, 3]. Recent advances in dairy technology have enabled the isolation of bioactive milk proteins from bovine milk in sufficient quantities for clinical investigations and, in some cases, addition to commercially available infant formulas [2].
Herein, the current evidence on HM protein composition and bioactivity of HM proteins will be reviewed, with a focus on lactoferrin, OPN, and the MFGM [4]. Lactoferrin is a non-heme iron-binding protein that has been shown to beneficially impact iron absorption in the breastfed infant and exert bacteriostatic effects. In the piglet model, bovine lactoferrin stimulated intestinal cell proliferation. In randomized controlled clinical trials, bovine lactoferrin reduced diarrhea and respiratory illnesses in term infants and sepsis and necrotizing enterocolitis in preterm infants [5]. OPN is an acidic, glycosylated, and highly phosphorylated protein. It interacts with cell surface integrins and the CD44 receptor to influence biomineralization, tissue remodeling, and immune regulation. Bovine OPN supplemented to formula at the concentration present in HM changed intestinal gene expression in rhesus monkeys to be more similar to breastfed monkeys. In a randomized controlled clinical trial, bovine OPN reduced fever incidence and serum TNF-a concentrations [4]. Lastly, MFGM is the triple membrane system that encapsulates milk fat. It contains cellular components, including cholesterol, glycerol phospholipids, sphingolipids, and proteins, including mucin 1, butyrophilin, CD36, adipophilin, and lactadherin. These bioactive components contribute to the antiviral and antibacterial activities of MFGM. In randomized controlled clinical trials, MFGM from bovine milk reduced diarrhea, fever, and antipyretic use and increased IQ [4]. In summary, HM contains many bioactive proteins that act independently and synergistically to

**Fig. 1. Biological functions of human milk proteins.**
provide multilayer defense against infection, as well as stimulate intestinal and cognitive development and shape the microbiome. Purification of bioactive proteins from bovine milk have allowed clinical trials in infants and will ultimately enable modifications in infant formula composition to narrow the differences in health outcomes between breastfed and formula-fed infants.

**References**

Early-Life Nutrition, Growth Trajectories, and Long-Term Outcome

Ferdinand Haschke, Christoph Binder, Mercedes Huber-Dangl, and Nadja Haiden

Introduction

It is well established that nutrition during the first 1,000 days can have a long-term effect on growth, metabolic outcome, and long-term health [1, 2]. We review long-term anthropometric follow-up of children with risk of later morbidity: (a) very-low-birthweight (VLBW) infants who have birthweights <10% percentile of weight and receive fortified breast milk, (b) infants from developing countries who are breastfed according to the present recommendations but have low birthweight and birth length, and (c) children from developed countries who were enrolled in randomized controlled trials (RCTs) to test if breastfeeding and low-protein formulas can prevent from rapid weight gain and childhood obesity.

VLBW Infants

Following international recommendations for nutrition of VLBW infants [3] (birthweight <1,500 g; <32 weeks of gestation) now contributes to better postnatal growth which should be parallel to a percentile line of intrauterine growth charts. The segment of VLBW infants which have a birthweight below the 10th percentile for gestational age (“born too small”) includes growth-restricted VLBW infants who are born constitutionally small (SGA) and VLBW infants with intrauterine growth restriction (IUGR) which is caused by a complex antenatal pathology [4]. For VLBW infants who are “born too small” ESPGHAN recommends an “enhanced nutrients strategy” which provides extra nutrients up to 52 weeks [3]. Actually, reliable data are lacking to guarantee that the recommended “enhanced nutrients strategy” is safe and effective for both SGA and IUGR infants in terms of long-term growth, i.e. no increased risk of persisting postnatal malnutrition as well as of later obesity, diabetes type 2, and cardiovascular events. We investigated the impact of the “enhanced
nutrients strategy” up to 52 weeks of postconceptional age on growth of VLBW infants (SGA with no genetic defects, malformations, intrauterine infections, \(n = 31\); IUGR with pathological ultrasound measurements \([4]\), \(n = 127\)). Mean birthweights of SGA and IUGR infants were 600 and 688 g (ns), and mean gestational ages were 25 and 29 weeks (<0.001), respectively. Enteral feeding of all infants started with breast milk that was then fortified with a human milk fortifier. At discharge, 68% of the infants still received breast milk. IUGR infants showed low weight with downwards crossing of weight percentiles between discharge and 3 months (corrected for GA). Mean weight of the SGA infants crossed the 10th percentile of the WHO standards of weight already at 6 months corrected for GA. A longitudinal analysis indicated higher weight of the SGA group between 3 and 24 months corrected for GA \((p < 0.05)\). BMI of both groups was similar during the observation period. Our data question the ESPGHAN approach \([3]\) that there is no need to develop separate nutrition guidelines for VLBW who are SGA or IUGR, but randomized controlled studies which include body composition and metabolic outcome measurements are necessary to prove the preliminary findings.

Breastfed Infants from Developing Countries – Stunting

The associations of breastfeeding with growth and health in developing countries can be studied by repeatedly analyzing DHS (demographic health surveys; US) datasets that provide information on nutrition, growth, and health. We reviewed data of more than 130,000 infants and small children (0–6, 6–12, 12–24 months) from 20 developing countries that were collected at least twice at intervals of 5–10 years during the last 2 decades \([5, \text{and unpubl. data}]\). Exclusive breastfeeding was associated with significantly higher weight, length, and lower probability of stunting, wasting, and infections. DHS data of infants between 6 and 24 months also reflect the influence of low-quality complementary feedings and poor environmental conditions in developing countries, which contribute to the high stunting and wasting rates. Growth trajectories from 2 well-controlled African cohorts \([6, 7]\) with strong breastfeeding support showed the importance of maternal stature, nutrition, and health as well as maternal nutrition before conception and during pregnancy: growth trajectories of infants who were in the top 10th percentile segment of length at birth grew almost according to the WHO standards until 2 years. However, those infants in the bottom 10th percentile segment at birth (i.e., newborns with disturbed intrauterine growth) showed poor growth and had mean length at 2 years that was below the \(-2\ z\)-score of the WHO standards. In addition to breastfeeding support, future key targets should be to improve nutrition
of adolescent girls, young women, and during pregnancy. Height catch-up in young children, even in the absence of external nutritional interventions, clearly contradicts the widely held impression that a window of opportunity closes at 24 months of age. The extent of catch-up after 24 months is highly context specific and presumably reflects the availability of foods, food-consumption patterns, the composition of diets, and the prevailing burden of infections (especially those affecting gastrointestinal function).

**Is Low-Protein Intake during the Breastfeeding Period and Beyond a Factor That Contributes to Prevention of Obesity?**

Infants fed traditional high-protein formulas have higher weight than breastfed infants at least until 24 months. RCTs indicate that infants receiving new low-protein follow-up formulas have lower weight gain during the first 12 months than infants receiving high-protein formulas [8–11]. Follow-up of clinical trials until 5–6 years indicates that children who were on low-protein formulas during the first year have BMIs similar to children who were breastfed [12, 13]. During longitudinal body composition follow-up, we found that percentage of body fat is more rapidly decreasing between 6 and 60 months, if children had been exclusively breastfed for 4–6 months or had been fed low-protein formulas until 12 months [13]. Children who received higher protein formulas during infancy showed only marginal decrease in percentage body fat until 60 months ($p < 0.05$). An RCT indicates that higher protein intake during infancy results in significantly higher BMI at 72 months and a higher percentage of childhood obesity [12]. A longitudinal cohort study that reflects the French childhood population [14] indicates that higher protein intake (>15% of calories) is associated with higher BMI during school age, adolescence, and young adulthood.

**Conclusions**

VLBW infants who are IUGR show low weight gain after discharge from hospital when they receive fortified breast milk. RCTs are necessary to confirm the results of our cohort study and to test new fortification strategies of breast milk. Exclusive breastfeeding is important to prevent infants from stunting in developing countries. Further preventive measures include nutritional supplementation of young women before and during pregnancy, promotion of breastfeeding and improvement of quality of complementary foods. RCTs which include follow-up of growth and
Body composition during childhood indicate that breastfeeding and the use of low-protein formulas can contribute to prevention of rapid weight gain during infancy and childhood obesity.

References

Brain development in the first years of life is the most dynamic and perhaps the most important phase of brain maturation [1]. Brain development can potentially be categorized into seven basic stages [2], including neurogenesis, cell migration, cell differentiation, dendrite and axonal growth, synaptogenesis, synaptic pruning, and myelogenesis, respectively. These complex and dynamic cellular processes set the foundation for the remarkable cognitive development and maturation during the first years of life. Any adverse effects leading to deviation from these well-orchestrated processes could result in life-long impacts on the health and development of our brain. It is widely recognized that adequate nutrition is necessary for normal brain development during pregnancy and infancy; particular nutrients most likely affect distinct aspects of brain development. The critical dosage windows and time frames for various nutrients needed at different stages of brain development are likely dissimilar. Long-chain polyunsaturated fatty acids are essential for neurogenesis and synaptogenesis, affecting pre- and postnatal brain development. Sphingomyelin is crucial for white matter myelination, which undergoes rapid developmental processes from late third trimester throughout the first years of life. It should also be recognized that different nutrients could contribute to similar aspects of brain development, functioning in a complementary or synergistic manner. For example, iron, choline, and sphingomyelin play important roles in white matter myelination. Table 1 summarizes a list of key nutrients with established and/or emerging roles on different aspects of early brain development. Large amount of knowledge about nutrition and brain is from preclinical investigations and clinical manifestations of nutrient deficiencies in humans. It remains a challenge to identify potential associations between intakes of specific nutrients and early brain development in normal healthy population when behavioral and cognitive assessments are employed as the sole outcome measures. Behavioral assessments, although robust, suffer from relatively low sensitivity,
difficulties assessing higher-order brain functions, particularly during infancy, and lack of ability to provide insights into the neural substrates underlying brain functional maturation. In contrast, magnetic resonance imaging (MRI) is capable of providing detailed anatomical and functional information – an ideal tool to characterize brain functional development and elucidate the effects of early nutrition. However, MRI is highly

<table>
<thead>
<tr>
<th>Nutrients</th>
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<tbody>
<tr>
<td><strong>Minerals</strong></td>
<td></td>
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<tr>
<td>Iron</td>
<td>Myelination, neurotransmission, brain growth, cofactor for brain enzymes</td>
</tr>
<tr>
<td>Zinc</td>
<td>Neurogenesis, neuron maturation and migration, cofactor for &gt;200 enzymes</td>
</tr>
<tr>
<td>Selenium</td>
<td>Component of selenoproteins in brain, antioxidants</td>
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<tr>
<td>Iodine</td>
<td>Neuron differentiation and maturation, myelination, neurotransmission</td>
</tr>
<tr>
<td>Copper</td>
<td>Neurotransmission, brain energy metabolism, antioxidant</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Brain energy metabolism, myelination, neurotransmission</td>
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<tr>
<td><strong>Lipids</strong></td>
<td></td>
</tr>
<tr>
<td>LCPUFA (DHA, ARA)</td>
<td>Neurogenesis and growth, synaptogenesis, two major lipids of gray matter</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>Major component of neuronal membrane, precursor for key second messengers</td>
</tr>
<tr>
<td>Sphingomyelin</td>
<td>Myelination, major component of myelin sheath</td>
</tr>
<tr>
<td>Gangliosides</td>
<td>Component of neuronal membrane, signal transduction</td>
</tr>
<tr>
<td><strong>B vitamins</strong></td>
<td></td>
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<tr>
<td>Folate</td>
<td>Myelination, neural cell proliferation and differentiation, DNA biosynthesis</td>
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<tr>
<td>B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>Myelination, neural cell proliferation and differentiation, 1-carbon metabolism</td>
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<tr>
<td>Choline</td>
<td>Neurotransmitter synthesis, myelination, DNA methylation</td>
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<tr>
<td><strong>Carotenoids</strong></td>
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<tr>
<td>Lutein</td>
<td>Major carotenoid in brain, antioxidant</td>
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<tr>
<td><strong>Proteins</strong></td>
<td></td>
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<tr>
<td>Lactoferrin</td>
<td>Major iron-binding protein, major whey protein in human milk</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Breast milk protein important for immunity and emerging research suggests a role in myelination</td>
</tr>
<tr>
<td><strong>Carbohydrates</strong></td>
<td></td>
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<tr>
<td>Human milk oligosaccharides</td>
<td>Emerging roles on brain likely via the microbiota-gut-brain connection</td>
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sensitive to motion artifacts. Nonetheless, our team has developed strategies that enable imaging of typically developing children from birth to teens without sedation [3].

Table 2 provides the first quantitative evidence (cortical gray matter volume [GM], cortical thickness [CT], and brain surface area [SA]) of early brain structural development from a cohort of typically developing children with a dense sampling scheme during the first 6 years of life. The total GM is almost doubled by the 9th month, followed by a much slower growth pace after the first year of life. Concurrently, CT increases in year 1 and decreases starting from the 18th month. Finally, SA exhibits a similar growth trajectory as that of cortical GM volume during the first 6 years of life, suggesting that SA expansion may play an equally or even more important role when compared to CT during early brain development.

In addition to characterizing brain structural development, resting state fMRI has been employed to discern detailed functional maturation during the first years of life [4]. During year 1, the topologies of the sensorimotor and auditory networks are highly consistent with those observed in adults. In contrast, higher-order brain functional networks are more primitive. In addition, while the visual networks are topologically similar to adults, they undergo tremendous growth in year 1.

Together, the structural and functional MRI has provided highly critical and quantitative insights into early brain development and serves as an important stepping stone to rigorously determine the potential interplay between nutrient intakes and early brain development. More
recently, research regarding brain imaging, cognitive development, and nutrition has intersected in expanded interdisciplinary efforts to understand the gut-brain axis, which could further shed light on our understanding of the complex interaction between brain development and gut microbiome [5].

References

Early-Life Nutrition and Gut Immune Development

Lieke van den Elsen, Akila Rekima, and Valérie Verhasselt

Gut immune function conditions development of diseases that result from defects in immune regulation such as allergic and obesity-related disease [1]. As epidemiological studies support the developmental origin of health and disease, the deciphering of the critical factors modulating gut immune development should allow the advance of primary prevention strategies specifically adapted to the early-life immune system. Here, we will emphasize how nutrition can shape microbiota composition and metabolite production with immune-modulatory properties. We will also focus on the role of dietary compounds recently demonstrated to be essential in immune development and function such as dietary antigens, vitamin A, and aryl hydrocarbon receptor (AhR) ligands.

Microbiota is necessary for lymphoid tissue development and immune differentiation such as IgA secretion, regulation of IgE responses, and differentiation of T cells subsets [1]. Besides mode of delivery, nutrition is the key factor directing the early microbiota composition and function [2]. Breast milk contains viable bacteria that will contribute to the establishment of the neonatal microbiota, and maternal IgA will alter colonization patterns in the neonate. Breast milk also contains nutrients specific for the growth of commensals, i.e. human milk oligosaccharides (HMO), which stimulate the growth of bifidobacteria and affect their metabolic function. In animals fed solid food, Clostridia can metabolize dietary fibers into short-chain fatty acids (SCFA), while in breastfed neonates, SCFA are derived from HMO metabolized by bifidobacteria (Fig. 1a). The role of SCFA in gut immunity in preweaned mice has not been assessed yet. In young weaned mice, they were found to stimulate regulatory T cell expansion, IgA and mucus secretion, gut epithelium barrier function, ILC3 function, and induce resistance to food allergy and gut inflammatory disease [3] (Fig. 1a). Some commensals, such as Lactobacillus, metabolize tryptophan, an essential amino acid that is a common constituent of protein-based foods (Fig. 1b). The metabolites bind AhR expressed in ILC3 and stimulate the postnatal formation of isolated
lymphoid follicles and IL-22 secretion necessary for gut barrier function and protection from *Citrobacter* infection and colitis [3] (Fig. 1b). In breastfed infants, AhR ligands could originate from maternal microbiota or from maternal diet (Fig. 1b).

Mice studies have recently highlighted the regulatory function of diet-derived antigens in the small intestine [4] (Fig. 1c). In the human [5],

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(For legend see next page.)
food diversification in the first year of life was associated with decreased risk of allergies. The shaping of immune reactivity by induction of oral tolerance to specific antigens during the period of immune ontogeny may be possible in the case of egg (OVA) and peanut antigen. Additional TGF-β, vitamin A, and IgG from maternal milk were critical for tolerance induction towards OVA transferred through breast milk in rodents [6] (Fig. 1c). TGF-β is a growth factor for epithelium, and both vitamin A and IgG acted on antigen transfer through epithelium. Vitamin A also increased the function of dendritic cells involved in tolerance and Th1 differentiation (Fig. 1c). Our recent data showed that not all the antigens in breast milk induce oral tolerance. Antigen from house dust mite, Der p 1, is present in human breast milk and its presence increased the risk of allergy both in mice and in the humans. This stresses the need to identify how maternal milk factors could be modulated to counteract deleterious action of some allergens [6].

In conclusion, before weaning, the physiological food for mammals is providing the neonate with the factors necessary for immune Fig. 1. Impact of food on immune ontogeny. a Short-chain fatty acids (SCFAs) stimulate regulatory T-cell expansion, IgA, and mucus secretion, gut epithelium barrier function, antimicrobial peptide secretion (AMP), and ILC3 function and induce resistance to food allergy and gut inflammatory disease. Human milk oligosaccharides (HMO) are present in human milk and stimulate the growth of bifidobacteria that can metabolize HMO into SCFA. After weaning, metabolic function of bifidobacteria changes, and they become able to metabolize complex sugars from dietary fibers similarly to clostridia found in microbiota of older children. b Aryl hydrocarbon receptor (AhR) ligands bind to AhR receptor expressed on ILC3. They stimulate the postnatal formation of isolated lymphoid follicles (ILF) and IL-22 secretion necessary for gut barrier function and protection from Citrobacter infection and colitis. AhR ligands are found in cruciferous vegetables such as broccoli and cabbage. They can also be produced by some commensals, such as Lactobacillus, which metabolize tryptophan from protein-based foods into indole derivatives. In breastfed infants, AhR ligands can originate from maternal microbiota metabolites with maternal milk immunoglobulin helping in the transfer of these metabolites to the neonate. Breast milk contributes also to AhR-mediated immune ontogeny by stimulating the growth of Lactobacillus. c After weaning, antigens derived from solid food are necessary for populating the small intestine with induced Tregs. Tregs specific to dietary antigens can be induced by oral exposure. Before weaning, oral tolerance can be induced to antigens from maternal diet present in breast milk. This requires the presence of additional cofactors in breast milk such as TGF-β, vitamin A, and IgG. Vitamin A increases gut barrier function, the capacity of dendritic cells (DCs) to metabolize vitamin A into retinoic acid and Th1 differentiation. Antigens bound to IgG are better transported across the epithelium and induce FoxP3 Tregs that are responsible for potent and long-lasting tolerance. After weaning, Treg induction towards oral antigen is favored by SCFAs. These induce TGF-β secretion from epithelium and stimulate retinoic acid formation from vitamin A by DCs.
maturation, which the neonate would otherwise miss due to the lack of a diverse microbiota and solid food-derived molecules. Breast milk exposes the infant to a variety of food antigens, and it contains ligands that are critical for lymphoid tissue development and immune function such as AhR ligands and vitamin A. It provides HMO, as surrogates to fibers found in solid food, for commensals to produce SCFA. Breast milk also delivers a microbiota and food for commensal growth in the sterile neonate gut. After weaning, solid food-derived antigens and vitamins as well as food metabolites produced by the microbiota will continue to shape the immune system and dictate susceptibility to local and systemic immune-mediated disease.

References

Early-Life Nutrition and Microbiome Development

Erika Isolauri, Samuli Rautava, Seppo Salminen, and Maria Carmen Collado

Recent reports link clinical conditions, phenotypes alternating from inflammatory bowel disease, obesity, and allergic diseases to neurodevelopmental disorders, to aberrant gut microbiota composition [reviewed in 1]. This has led to a growing interest in host-microbe cross talk, characterizing the healthy microbiome and modifying its deviations at an early age. The rationale arises from the recognition of the intimate interrelationship between diet, immune system and microbiome, and the origins of human disease.

Before satisfactory preventive measures can be put in practice, important questions remain to be solved. First, we need more profound understanding of the complex mechanisms underlying these heterogeneous manifestations of immune-mediated and microbiome-associated chronic conditions. Second, long-term follow-up studies are required to determine whether the changes in the microbiome underlie the pathogenesis of noncommunicable diseases or are merely end results thereof, confronting the question of causality. This uncertainty notwithstanding, the complex and bidirectional interrelationship of the diet and the gut microbiota is becoming evident. Early exposures by the enteral route induce dynamic adaptive modifications in the microbiota composition and activity, which may carry long-term clinical impacts. Microbiota changes, again, control energy acquisition and storage and may contribute to gut immunological milieu; high-energy Western diet alters the microenvironment of the gut leading to propagation of the inflammatory tone and perturbation of gut barrier function and thereby to systemic low-grade inflammation [2, 3].

The cornerstone of prevention of noncommunicable diseases is breastfeeding [4]. Not only does it provide the infant with nutrients, it may also confer immunologic protection at the portal of entry where major load of antigens is encountered, the gut barrier. A delicate balance of stimulatory, even inflammatory, maturational signals, together with a myriad of anti-inflammatory compounds, is transferred from mother to
infant via breastfeeding. Human milk protective compounds also include specific oligosaccharides and fatty acids influencing early microbial colonization and gut barrier adherence of pathogens and other microbes, but also specific microbiota and molecules operating in host-microbe interaction.

Breastfeeding provides several health benefits that are likely to be caused by promotion of age-appropriate and environment-adjusted gut colonization. There is abundant evidence that breast milk complements the microbiota transmission to the infant gut: the mother provides the infant with bifidobacteria, lactic acid bacteria, and other microbiota components in significant quantities during breastfeeding. Several active compounds of breast milk accomplish this progression. However, the microbes and other active compounds in breast milk strongly vary according to the mother’s health and weight gain during pregnancy, and the mode of delivery. In general, the infant’s probability of being colonized by bifidobacteria is lower when the mother has higher BMI, excessive weight gain.
during pregnancy, and the child is delivered via caesarean section, and higher when the mother is of normal weight, has notable bifidobacteria colonization in her own gut and breast milk and is breastfeeding (Fig. 1).

The model of early nutrition for future studies is the healthy breastfed infant that remains healthy in the long-term. Scientific interest is currently extending from the duration of breastfeeding to the composition of breast milk, and characterization of the key regulatory substances therein. Human milk, rich in bioactive compounds including health-promoting microbes and their optimal growth factors, human milk oligosaccharides, continues to afford tools to study diet-microbiota interactions for research aiming at reducing the risk of noncommunicable diseases.

References

Human Milk and Clinical Outcomes in Preterm Infants

Paula P. Meier

Human milk from the infant’s own mother (mother’s own milk; MOM) has been linked for decades with beneficial clinical outcomes for infants born prematurely, especially those born extremely preterm (<32 weeks of gestation) and very-low birthweight (VLBW; birthweight <1,500 g). The primary impact of MOM in this population is the reduction of potentially preventable morbidities including: necrotizing enterocolitis, late-onset sepsis (sepsis), bronchopulmonary dysplasia, severe retinopathy of prematurity, neurodevelopmental problems, and rehospitalization after discharge from the neonatal intensive care unit (NICU) [1]. Additionally, MOM supports adequate growth with exogenous fortification and clinical management of lipid variability in pumped and stored MOM. Recently, studies have examined the cost-effectiveness of MOM as a targeted NICU strategy to reduce the incidence and severity of these costly morbidities, which set the stage for lifelong neurodevelopmental and health problems and their associated costs. However, all studies are limited by the inability to randomly assign MOM feedings, so the most common research design is the observational cohort. Additional limitations in studies include: retrospective and/or secondary analyses of datasets that were not designed specifically to measure the impact of MOM, inconsistent and imprecise measurement of amounts of MOM and non-MOM received by the infant; inconsistent diagnoses of morbidities; lack of inclusion of minority infants who received MOM; older cohorts (1980–1990s) with different medical care and nutritional options; and combining MOM and donor human milk (DHM) feedings into a single human milk feeding measure.

The LOVE MOM cohort (Longitudinal Outcomes of Very Low Birthweight Infants Exposed to Mothers’ Own Milk; NIH: R010009; Meier PI) enrolled 430 VLBW infants (52% black, 27% Hispanic, 21% Caucasian) between 2008 and 2012, with the objective of determining the health outcomes and costs of differing doses and exposure periods of MOM feedings, while controlling for the limitations in previous studies. In this prospective cohort study, 98% of infants received
some MOM; mLs of MOM and formula (no DHM was used) were measured daily, and MOM dose was calculated both as a percentage of total enteral feedings and as a weight-adjusted (mL/kg/day) measure. Morbidities were diagnosed and validated independently by 2 neonatologists; cost data were actuarial rather than estimated, and propensity scoring was used to control for the risk of morbidities (confounders). Data revealed a dose-response relationship between higher amounts of MOM received during critical exposure periods during the NICU hospitalization and a reduction in the risk of specific morbidities and their costs (Table 1). These findings suggest that MOM functions by different mechanisms over the course of critical exposure periods to reduce the risk of potentially preventable morbidities and their associated costs in VLBW infants.

**References**

The first years of a child’s life are critical for growth and development, and despite decades of research, we still do not understand how infant diet shapes a child for both short- and long-term health. The link between food and health is complex, and although breastfeeding is known to have short- and long-term benefits, the relationship between food and the developing neonate is not understood, primarily because in the past there has been a lack of analytical tools. Indeed, most infant studies rely on crude measures of child health such as growth, and absence of obvious disease. While these assessments can reveal rudimentary associations between dietary components and lack of adverse outcomes in the short-term, they do not directly address the impact of food or food components on metabolic health that may have long-term consequences.

Making matters more complex, analysis of food is not trivial. Although decades of research have gone into studying human milk, most research has focused on studying proteins, lipids, and micronutrients. It is now recognized that there are other factors in milk that may be important for infant health, including small-molecule metabolites that include a unique class of sugars known as oligosaccharides. Human milk oligosaccharides, which are complex in structure, act as both food for beneficial bacteria and decoys for pathogens [1], and it is now being shown that they can help build the immune system through modulating CD14 expression and altering plasma cytokine levels [2, 3]. Additionally, there are other metabolites present in milk, and their function is not fully understood, although their expression appears to be controlled through the mammary gland as well [4], and they may have important consequences for the developing neonate. Through the development of modern nuclear magnetic resonance- and mass spectrometry-based metabolomics techniques, we are now in an era where we can measure these small molecules in food, and this will help us understand how food impacts health in an unprecedented way.

Analysis of the infant metabolome has led to important revelations regarding how infant diet impacts development. Breastfed infants
have been shown to have lower levels of plasma branched-chain amino acids (isoleucine, leucine, and valine), and urea, as well as higher levels of ketone bodies (acetone), acetate, and myo-inositol [5]. Additionally, breastfed infants have lower insulin levels than their formula-fed counterparts 2 h after feeding [5]. High levels of serum branched-chain amino acids and/or insulin activates mechanistic target of rapamycin (mTOR), a serine/threonine kinase that is a master regulator of cell metabolism. mTOR Complex 1 (mTORC1) signaling is particularly important for the control of growth and metabolism of bone, skeletal muscle, the central nervous system, the gastrointestinal tract, blood cells, and other organs. For formula-fed infants, enhanced activation of this pathway may have lasting impacts on overall metabolism and potentially health.

More study of human milk and infant metabolism that incorporates metabolic phenotype (measured through the metabolome of blood, urine, and feces), gut microbial composition and function, as well as genetic (and epigenetic) data will help us understand the purpose of specific milk components, the individual responses to diet, as well as how diet and genetics work together with the gut ecosystem to guide cognitive and metabolic development.

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Human Milk Oligosaccharides: Next-Generation Functions and Questions

Lars Bode

The past decade has experienced an immense increase in research on human milk oligosaccharides (HMOs), mostly driven by (a) advances in high-throughput glycan analysis and (b) large-scale glycan synthesis as well as (c) the advent of modern microbiome research.

Advances in high-throughput glycan analysis have enabled the research community to analyze HMO composition in hundreds and sometimes thousands of milk samples from large mother-infant cohorts to investigate associations between maternal factors and HMO composition as well as between HMO composition and infant outcomes. However, the identified associations cannot prove cause-and-effect relationships. Cohesive and consistent results from suitable preclinical in vitro, tissue culture and animal models, human cohort associations, as well as randomized controlled trials (RCTs) will be required to make conclusive claims about specific HMO functions. As an example from our own work, we identified a specific HMO called disialyllacto-N-tetraose (DSLNT) that reduces incidence and severity of necrotizing enterocolitis (NEC) in a rodent model [1]. While the results are encouraging, the validity of data from available preclinical NEC models in rodents or piglets is limited [2]. Animals are exposed to external hypoxic and/or hypothermic insults that are rather artificial, and the use of animals itself is a limitation due to interspecies differences in gastrointestinal development, anatomy, and physiology. Thus, advancing a potential therapeutic like DSLNT from controversial preclinical models to clinical treatment trials carries a tremendous risk of failure. However, in parallel, we conducted a multicenter clinical cohort study on 200 mothers and their preterm, very low-birthweight infants that were predominantly human milk-fed and showed that infants who developed NEC received less DSLNT with the milk than infants who did not develop NEC [3]. The latter results do not prove cause-and-effect, but the combination of preclinical testing and human cohort associations raises the confidence towards clinical application. Future studies in the preclinical model as well as in tissue culture
are going to help us elucidate the underlying mechanism, and a carefully
designed RCT is going to be needed to ultimately prove the use of DSLNT in NEC prevention.

Advances in glycan synthesis have made individual HMOs available
for research as well as commercial application. Chemoenzymatic synthe-
sis is extending our repertoire of available HMOs at smaller scale for in
vitro and preclinical research [4]. The help of bioengineered microbes
allows the synthesis of individual HMOs at large scale for commercial
application [5]. However, knowledge about specific functions and poten-
tial adverse effects of individual HMOs remains very limited at best.
Human milk contains a personalized mixture of hundred or more dif-
ferent and structurally distinct HMOs, which raises questions whether
the application of single individual HMOs instead of complex mixtures
causes imbalances in infant gut microbial communities or in the infant
immune system with potential short- and long-term health consequences.

Advances in microbiome research and the associated analytical and
bioinformatics tools further sparked an interest in HMOs. The compo-
sition of microbial communities in the gut and other organs and their
metabolic and functional capabilities have been associated with numer-
ous conditions, including obesity, inflammatory bowel disease, autism,
asthma, and allergies – to only name a few. The concept that HMOs
help shape microbial communities early on in life and affect short- and
long-term health is indeed striking. Future research is going to apply a
combination of preclinical and clinical studies to systematically elucidate
HMO structure-function relationships and identify whether individual
HMOs like DSLNT or mixtures of HMOs (the way they naturally occur in
human milk) provide short- or long-term benefits to infants and poten-
tially adults.

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Significantly premature and low-birthweight babies have acute challenges for survival, largely due to limited development of their lungs and gut. Furthermore, disruption to the timing of setting developmental clocks in the neonate often results in increased frequency of mature-onset disease, and this is exacerbated if growth rates are accelerated too aggressively [1]. The cost to manage these babies in hospitals is considerable, and there is an increased prevalence of the problem in the developing world. Mothers often cannot breastfeed and the only option available is providing either formula or pasteurized donor milk to improve the growth of these babies. New approaches are required to manage the early stages of treatment, particularly a focus on development of tissues without an accompanying large increment in growth.

New innovative approaches for therapy are emerging with the opportunity to exploit the unusual reproductive strategy of the tammar wallaby, an Australian marsupial. This animal has a short 26-day gestation which is associated with a poorly developed placenta, and gives birth to an altricial young that is equivalent to a mid-late pregnant human embryo. Lactation extends to 300 days, and consequently there is greater investment in postnatal development of the young.

The pouch young are born with immature organs, and during early lactation the mother limits growth while organs necessary for their survival such as gut, lung, lymphoid tissues, and nervous system (including brain and spinal cord) are rapidly developed [2]. The tammar neonate remains in the pouch and attached to the teat for 100 days, and the mother progressively changes the composition of the milk to deliver signals for this development; this closely resembles the relationship between the human fetus and placenta. Fostering experiments demonstrated that transferring the early-phase tammar pouch young to a later-phase lactating tammar accelerated the growth and physical development of pouch young [3] (Fig. 1C). Therefore, examining timed delivery of bioactives
Fig. 1. a The tammar as a model system for premature and low-birthweight babies. The tammar young is 6 days of age, the human neonate is 26 weeks of age. b Tammar wallaby lactation strategy. Progressive changes in milk composition and growth of the young during the three phases of the lactation cycle in the tammar wallaby. Total protein concentration does not change significantly during early lactation, but there is a progressive change in the kinds of proteins secreted. c Fostered pouch young. Pouch young at day 120 of age were either cross-fostered to host mothers at day 170 of lactation or retained on mothers at day 120 of lactation. After 50 days, both pouch young were removed. The more mature animal (shown on the right) was fostered to a mother at a more advanced stage of lactation.
in the milk may provide a better understanding of the signaling program of the placenta, and other tissues, required for normal eutherian development.

The composition of milk in eutherians does not change significantly during lactation apart from the transition from colostrum to mature milk. However, the signaling factors from the human placenta, amniotic fluid, and potentially colostrum involved in the development of the fetus/neonate are most likely delivered in the milk of marsupials in the early phase of lactation. For example, the lung in newborn marsupials is so immature at birth, the neonate respires through the skin for the first 2 weeks. Studies using in vitro models have shown that milk collected from marsupials during early lactation (day 20–100), but not late lactation (day 100–300) stimulated proliferation and differentiation of cultured whole lung from...
mouse embryos, and these signaling molecules were directed to differentiation of both lung epithelial and mesenchymal cells [4]. This temporal delivery of bioactivity provides a window to search for the signaling molecules in the milk (Fig. 2).

A second approach has exploited the comparison of databases of differentially expressed genes in the tammar mammary gland in early lactation, human milk, colostrum, placenta, and the amniote. A focus only on genes coding for secreted proteins has allowed for a comparison of potential protein-signaling molecules secreted by these tissues. This latter analysis has identified a number of proteins of interest in placenta and amniotic fluid, and unexpectedly identified signaling candidates in colostrum, prompting the need to reexamine the role of colostrum in development of both term and preterm babies.

Exploiting comparative approaches provides new options to understand the role of milk in acute and chronic development of the baby. Studies using the tammar wallaby may lead to a new range of human milk fortifiers that include bioactives to specifically target tissue development in the human neonate to improve outcomes for premature and low-birthweight babies with application in the developed and developing world.

References

Milk Lipids: A Complex Nutrient Delivery System

J. Bruce German

The evolutionary origin of lactation and the composition, structures, and functions of milk’s biopolymers illustrates that the Darwinian pressure on lactation selected for biopolymers with considerable structural complexity and discrete functions within the digestive system [1]. For example, complex sugar polymers, oligosaccharides, possess unique properties in guiding the growth of intestinal bacteria that are not possible by feeding their simple sugars; proteins exhibit enzymatic activities towards other milk components rendering those components both more digestible but also releasing biologically active products. To date, however, the most complex structure in mammalian milk, the fat globule, has not been effectively examined beyond its simple composition. The globules of milk are heterogeneous in size, composition and function, and new research tools and models are beginning to understand the mechanisms that control the assembly of globules in the mammary gland and the disassembly within the infant.

Assembly

Milk globules represent a unique biological particle class composed of a triglyceride core bounded by a phospholipid monolayer, assembled in the endoplasmic reticulum, and a complete bilayer structure enrobed around the globule by the mammary epithelial plasma membrane during globule secretion [2, 3]. The size, diversity, and composition of milk fat globules changes during lactation and as a function of genetics, diet, and mammary gland metabolism [4]. The size of fat globules is directed in part by the availability of specific precursor lipids [5]. Phosphatidyl choline and phosphatidyl ethanolamine compete for occupancy of the globule surface, and their distinct physical properties guide the fusion events that ultimately determine the size of globules as they form and are secreted into milk. The proportion of these two complex phospholipids is thus a vital determinant of globule assembly. Lipid trafficking within the
mammary epithelial cells is determined in part by available fatty acids and by mitochondria. These research breakthroughs not only provide a mechanistic understanding for these processes but offer the possibility to guide globule size, composition, and function with exogenous treatment [5].

Disassembly

Fat globules undergo complex disruption during digestion within the intestine of the infant. The importance of the digestion process has been implied by the discoveries of endogenous lipase enzymes within the milk itself that guide lipid digestion even in the absence of digestion capacity within early infants. Complex lipids interact with the aqueous phase to produce a diverse tableau of possible structures that exhibit considerable organization within the nanometer and micrometer length scales. The biological membrane is just one example of a three-dimensional lipid phase that forms spontaneously depending on the concentration and composition of lipids. As the lipid composition of fat globules changes within the intestine due to changes in the molecular structure of complex lipids resulting from hydrolysis, new three-dimensional phases/structures can form spontaneously. However, these phases are dynamic and change spontaneously as a function of concentration. Dynamic lipid structures have been ostensibly impossible to follow scientifically due to their ephemeral nature. High-intensity, coherent X-rays are formed by synchrotron accelerators. It is thus possible to introduce complex lipid mixtures into these beams and follow the structures formed in real time. Recent studies on the phase changes during lipolysis have demonstrated that the ensemble of complex lipids of mammalian milks forms distinct cubic structures in real time [6]. The cubic phase is of particular interest since it is a three-dimensional bicontinuous phase capable of dissolving and transporting both water-soluble and lipid-soluble components in all directions. The cubic phase presence has been linked to successful absorption of a variety of fat-soluble nutrients, yet it has not been possible previously to determine if milk lipids form cubic phases. Thus, the lipid globule constitutes the precursors for a complex, higher-order, structured delivery system that self-assembles within the infant’s intestine, facilitating absorption by the infant.

Composition

The composition of mammalian milk lipids is a perplexingly sensitive process. Unlike the other biopolymer classes (proteins, glycans, polynucleotides), lipid composition is significantly altered by the composition of fatty acids within the maternal diet. As a result of the widespread observations of the environmental influences on milk composition, considerable
research has focused on this aspect. Much less interest has focused on the fatty acids that are synthesized by the mammary gland itself. Some fatty acids are relatively constant in milk across species, and new research is beginning to reveal functions of these fatty acids beyond simple fuel provision. The most abundant single fatty acid in milk, palmitic acid, has been identified as a potent ligand for the PGC-1β transcription coactivator within the liver [7]. This nuclear regulator orchestrates the transcription of the complex machinery necessary to synthesize and assemble very-low-density lipoproteins within the liver, thus guiding not only liver lipid secretion but whole-body energy metabolism. The fatty acid derivative of desaturation of palmitic acid, palmitoleic acid, has been identified as possessing an alternative signaling function. Characterized as the first of a class of lipid signaling lipids “lipokines,” palmitoleic acid controls hepatic gluconeogenesis, lipid uptake in the muscle, and potentially food intake systemically [8]. The presence of these fatty acids within milk suggests that various aspects of fuel metabolism within the infant are under direct control of the composition of milk’s constitutive fatty acids.

This growing body of evidence argues for a broader view of milk composition that includes the complex structures of large biopolymers, their structures as ensembles, their distinct activities within the milk as it is digested, and the influence of this structural dimension across the nanometer and micrometer length scales on the health value of milk within the entire diet.

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