



The 90th Nestlé Nutrition Institute Workshop

**Human milk:
composition, clinical benefits
and future opportunities**

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90th NNIW SWITZERLAND

FORWARD

Human milk presents the optimal nutrition for infants and is key to sustaining health and building the foundation for growth and cognitive development. The World Health Organization (WHO) recommends that infants should be exclusively breastfed for the first 6 months of life and subsequently receive suitable complementary foods while breastfeeding continues up to 24 months of age or beyond.

Rapidly-advancing technology has allowed us a closer look at the different components of human milk and shed light on their biological effects on growth, metabolism, cognition, and immunity. Yet researchers face many challenges in their quest to unravel its complexities. An understanding of human milk is inextricably linked to an understanding of the biology of the growing infant. Any clinical study that aims to elucidate the effects of a specific element in human milk must overcome the double hurdle of design and outcome: how can we test such a complex substance or extract a meaningful endpoint from the intricacies of infant development? Success relies in part on obtaining a cohesive body of *in vitro*, *in vivo*, and clinical data.

The 90th Nestlé Nutrition Institute Workshop brought together the world's experts on human milk, chaired by Professors Sharon M. Donovan (Professor and Melissa M. Noel Endowed Chair in Nutrition and Health, Department of Food Science and Human Nutrition, Carl R. Woese Institute for Genomic Biology, University of Illinois), J. Bruce German (Director, Foods for Health Institute, University of California, Davis), Bo Lönnerdal (Distinguished Professor Emeritus, Department of Nutrition and Internal Medicine, University of California, Davis) and Alan Lucas (MRC Clinical Research Professor and Head of the Childhood Nutrition Centre, Institute of Child Health, University College London). The four sessions in the workshop touched on the full spectrum of our knowledge of human milk, from the history and mechanics of breastfeeding, its physiological effects, to the new surprises revealed by metabolomics and comparative biology.

Dr Natalia Wagemans
Head of Nestlé Nutrition Institute
Switzerland

SESSION 1

State of Breastfeeding in the World

Chairperson: Alan Lucas (University College London)



The first session began with Professor Ruth Lawrence's historical overview of the rise and fall of breastfeeding, up to its renaissance as today's gold standard for infant feeding. A product of 200 million years of mammalian evolution, human milk is the benchmark for optimal infant nutrition and has wide-ranging clinical benefits. Next, Professor Lucas tackled the key paradigms that form the foundation of infant feeding research, confronting the challenge of interpreting a large body of data in order to understand the infant's nutritional needs. Finally, effective breastfeeding management relies on a knowledge of how the infant extracts milk from the breast. Dr. Michael Woolridge ended the session with a talk on the functional biomechanics of breastfeeding.

Breastfeeding in Medicine - A Historical Perspective

Ruth Lawrence (University of Rochester School of Medicine) described how the role of breastfeeding has evolved throughout the ages. From ancient times up to the Industrial Revolution, breastfeeding was the norm for feeding infants immediately after birth and throughout the first years of life. In the 19th and 20th centuries, however, a sea change came about in infant feeding practices. The period of social upheaval that followed the Industrial Revolution caused many women to enter the workforce, a fact that precluded breastfeeding for many mothers. For several years, the scientists of the era had been looking for substitutes for mother's milk and some advocated the addition of sugar and water to cow's milk.

In the meantime women were exposed to conflicting discourses on the best method for feeding their infants, in publications such as *The Ladies Home Journal*. As more evidence accumulated, scientists became aware of the higher infant mortality rate that was associated with some artificial milk substitutes including those based on cow's milk or soy. Alongside the higher infant mortality rate, some infants grew faster and became obese. Clearly, not all milk substitutes were alike.

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There is more to learn and much to respect about the handiwork of Mother Nature. ”

Ruyh Lawrence

Today, breastfeeding has seen a rebirth. The importance of breastfeeding was proclaimed in the Innocenti Declaration, with the goal of protecting and supporting breastfeeding as a key infant feeding practice worldwide. Scientists now use human breast milk as a reference, not only for the design of appropriate milk substitutes but also to gain a greater understanding of how breast milk influences infant growth and development. Breastfeeding has been restored and must be maintained as the gold standard worldwide, for generations to come.

Breastfeeding and Health - A Biological Perspective

Alan Lucas (University College London) presented a critical evaluation of three key paradigms of breastfeeding, identifying the flaws in order to build a more solid evidence base to support infant feeding research. The three key paradigms are: 1) The product of 200 million years of mammalian evolution, human milk is ideally adapted for babies; 2) The composition of human milk should be seen as the gold standard for meeting the infant's nutritional requirements, and 3) Human milk has numerous clinical benefits for the infant.

First, there is a mismatch between human genes and the rapidly changing environment (evolutionary discordance), suggesting that human milk may not be perfectly adapted for all circumstances. Evidence for this arises from the study of micronutrient deficiencies, including deficiencies in vitamins K, D, iron, and omega-3 fatty acids, as well as disorders such as cardiovascular disease.

Can human milk composition be used as the reference for meeting an infant's nutritional requirements? Past literature provides only incomplete snapshots of breast milk composition, leading to conflicting data and misleading conclusions. This also resulted in the incorrect design of early infant formulas. Improved techniques for determination of breast milk nutrient content (for instance using stable isotopes) are important for generating more accurate reference data.

Finally, most of the studies that claim to show the clinical benefits of human milk are observational and potentially confounded. But randomized controlled trials (RCTs) of breastfeeding versus formula feeding are fraught with ethical and methodological concerns and rarely done. A useful model is the pre-term infant, as there are numerous studies (including RCTs) which compare human milk versus cow's milk exposure. Long-term programmed effects have been seen in terms of cognition, brain structure, heart, lung and bone development,

as well as in disease risk factors. These data add much weight to the evidence that can be extrapolated to full-term infants, that human milk feeding in early life may fundamentally and permanently change the biology, health and developmental outcomes of the organism.

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Human milk feeding in early life may fundamentally and permanently change the biology, health and developmental outcomes of the organism. ”

Alan Lucas

Physiological Basis/ Mechanics of Breastfeeding

Michael Woolridge (University College London) explained the principles of positioning and attachment and why they are critical factors that underlie breastfeeding success.

Infants extract milk from the breast by a combination of baseline suction, compression and relaxation of the baby's jaws against the breast, along with rhythmical waves of pressure applied by the baby's tongue. This principle is the cornerstone of the WHO/UNICEF training model, which focuses on optimizing the positioning and attachment of the baby at the breast, in order to maximize the effectiveness of milk transfer.

New insights have been gained from bioengineering studies, obtained using the latest ultrasound equipment alongside modeling of the infant's sucking action on the milk duct system.

A key novel finding is that the infant can also generate localized, added suction with its tongue to enhance milk transfer. This secondary mechanism is used together with the main process of peristaltic expression by the tongue to extract breast milk.

Nevertheless, the findings of bioengineering studies have several limitations. Key physical assumptions have to be made in order to simplify the modeling process. Some of these are known to be incorrect, for example, the milk duct walls are 'rigid'. Other ways in which the modeling process deviates from known physiology include: i) the assumption that negative suction pressure is the primary force, with no contribution from the progressive peristaltic pressure exerted by the baby's tongue, and ii) the milk duct system remains patent throughout a feed, ignoring the occlusive impact of the baby's jaw closure with each suck. The inclusion of any one of these physiological processes could radically alter the conclusions from modeling.

What are the clinical implications of these findings? The pivotal role played by the infant's peristaltic tongue movements is essential to effective breastfeeding, and underscores the importance of positioning and attachment to breastfeeding success. This in turn enables the infant to maximize the transfer of nutrients, particularly breast milk fat.

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Understanding how a baby extracts milk from the breast is the vital cornerstone to practicing sound, effective breastfeeding management. ”

Michael Woolridge

SESSION 2

Milk Composition and Physiological Benefits

Chairperson: Bo Lönnerdal (University of California, Davis)

Session II investigated the main components of human milk, showcasing the unique aspects and bioactive properties. A common theme in this session was the great variation in many of the bioactive components including essential fatty acids, fat- and water-soluble vitamins. Although we know that these micronutrients are essential for the maintenance of cellular growth and function, there is little hard data to guide interventions. Studies in specific populations, such as pre-term infants and those with deficiencies, provide a useful insight into the roles of these important components of human milk. Professor Olle Hernell began the session with a look

at the milk fat globule membrane. This was followed by Dr. Norbert Sprenger's overview of the non-digestible human milk oligosaccharides. Professor Ardythe Morrow delved into the fatty acids and fat-soluble vitamins, while Professor Lindsay Allen continued with the water-soluble vitamins. Professor Bo Lönnerdal put the spotlight on human milk exosomes, before Professor Sharon Donovan closed the session by reviewing the proteins in human milk.



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

The milk fat globule membrane (MFGM) is a biologically active fraction of milk that contains a large proportion of critical milk phospholipids. Olle Hernell (Umeå University) reviews the clinical evidence on the role of MFGM concentrates and the outcomes in formula-fed infants.

Compared to breastfed infants, formula-fed infants have a lower intake of MFGM because the MFGM fraction is frequently discarded with the milk fat when this is replaced by vegetable oils as the fat source in infant formulas. Therefore, MFGM supplementation is of special relevance to formula-fed infants.

Clinical studies on supplementing the diet of infants and children with bovine MFGM have shown promising results with respect to growth, neurodevelopment and immunity. However, the evidence base to support MFGM supplementation is still limited. First, the number of published studies on MFGM in infants and children is relatively small. Furthermore, the study designs are heterogeneous: different MFGM concentrates have been administered for varying lengths of time, the target populations are of different ages and the primary outcomes vary.

With these limitations in mind, the findings from various trials suggest that MFGM supplementation may have protective effects on immunity, reducing the incidence and duration of infections. In addition, benefits have been seen in cognitive and motor outcomes, including intelligence scores and hand-eye coordination. No serious adverse effects have been reported and MFGM supplementation appears to be safe from the first week of life in term infants. Infant formulas supplemented with bovine MFGM concentrates have already been launched in many markets, but further high quality clinical trials are needed to conclusively establish the health benefits.

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Formula-fed infants are of special interest with respect to MFGM supplementation since they have lower intake of MFGM components as compared to breastfed infants ”

Bo Lönnerdal

Human Milk Oligosaccharides: Factors Affecting their Composition and their Physiological Significance

Norbert Sprenger (Nestlé Research Center) focused on the third largest solid component of breastmilk: the non-digestible human milk oligosaccharides (HMO).

HMO are a diverse group of soluble oligosaccharides that are carbohydrate polymers formed by elongation of the milk sugar lactose by galactose, N-acetyl-glucosamine, fucose and sialic acid. The prevalence and composition of HMO varies greatly, depending on the maternal fucosyltransferases FUT2 and FUT3, and according to lactation stage. Maternal nutritional and health status may also influence HMO composition in breastmilk.

What are the functions of HMO? Data from observational and experimental studies suggest several key biological roles for HMO, including i) establishment of the early life microbiota dominated by bifidobacteria, ii) resistance to pathogens, and iii) contribution towards intestinal mucosal barrier and immunity, thereby conferring immune protection.

Clinical intervention trials with infant formula supplemented with 1 HMO (2'FL [2'Fucosyllactose]) or 2 HMOs (2'FL with LNnT [Lacto-N-*neotetraose*]), demonstrated that they supported age-appropriate growth and were well tolerated. Use of an infant formula containing 2 HMOs was also associated with fewer lower respiratory tract illnesses and reduced the need for related medication such as antibiotics and antipyretics, during the first year of life. Furthermore, this formula shifted the global microbiota profile towards that of breastfed infants. Interestingly, infants with a microbiota community structure typical of control formula-fed infants had a two-fold higher risk of antibiotics usage during the first year of life, compared to those with a microbiota community typical for breastfed infants. Altogether, clinical observational studies corroborated by preclinical experimental data and clinical intervention trials support a role for specific HMO in immune protection.

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Together, clinical observational studies corroborated by preclinical experimental data and clinical intervention trials support a role for specific HMO in immune protection leading to reduced use of antibiotics during the first year of life ”

Norbert Sprenger

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

The lipid fraction of human milk is not only the most important source of dietary energy, but also contains a large number of bioactive components necessary for the developing infant. Ardythe Morrow (University of Cincinnati College of Medicine) gave further insight into this little-studied aspect of human milk.

The major constituents of the lipid fraction are fatty acids and fat-soluble vitamins. These play a critical role in neurodevelopment, cardiovascular health, and immune regulation. The three most abundant fatty acids of human milk – oleic, palmitic and linoleic acid – comprise about two-thirds of the fatty acid fraction. Although there are core fatty acids present throughout diverse global populations, fatty acid composition is otherwise highly variable across populations and between mothers, depending on diet and genetics. Some of these variable fatty acids include docosahexaenoic acid (DHA) and other omega-3 fatty acids, the trans-fatty acids, and branched-chain fatty acids. In addition, there are also differences in the levels of fat-soluble vitamins. Of note, human milk typically has limited levels of vitamins D and K.

What are the effects of these variations on infant health? Little is known about the health and long-term impact of these natural variations in human milk. The strongest evidence comes from studies in preterm infants. The milk of mothers who deliver preterm may be lacking in omega-3 fatty acids and fat-soluble vitamins. Maternal supplementation with preformed DHA may therefore present an important intervention in this setting. The global public health consensus is to supplement all infants with vitamin D for prevention of nutritional rickets. However, recent data indicates that vitamin D supplementation of lactating women can also result in sufficient levels of vitamin D in breast milk. The inherently low level of vitamin K in human milk is typically compensated for by endogenous bacterial synthesis or by direct vitamin K administration to high-risk infants. Nutritionally needy populations (including pre-term infants, and pregnant women and infants from low-resource areas) are a key target for research and intervention.



The impact of differences in human milk fatty acid profile on infant health is understudied and an important domain of research ”

Norbert Sprenger

Water-Soluble Vitamins in Breast Milk: Factors Affecting their Concentrations and their Physiological Significance

Water-soluble vitamins are essential for the 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. Lindsay Allen (University of California, Davis) described the roles of these vitamins and the natural fluctuations in their levels during the course of lactation.

There are natural changes in the concentrations of water-soluble vitamins in human milk over the course of lactation. While vitamins B1 (thiamin), B3 (niacin), and B5 (pantothenic acid) increase throughout the course of lactation, vitamins 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 of increasing and decreasing concentrations, until its levels stabilize in late lactation.

The concentrations of most of the water-soluble vitamins are influenced by maternal status and/or supplementation. Other factors affecting the concentrations of some water-soluble vitamins in human milk include parity, preterm delivery, diurnal variation, smoking, medication intake, and maternal illness.

Lack of high-quality studies is the key hurdle to setting guidelines that outline the recommended intakes of these vitamins for infants and lactating mothers. Allen's team has developed more efficient, validated methods that enable the measurement of most of the B vitamins and their vitamers simultaneously in small volumes of milk. The outcomes of these studies are two-fold. First, they reveal large differences in concentrations among various populations around the world. Second, they provide data on the effects of multiple micronutrient supplements on milk vitamins. One important question that remains is how to define a "low" value. This question is the goal of the Mothers, Infants and Lactation Quality (MILQ) study, which aims to evaluate the concentrations of vitamins (and other nutrients) in milk from well-nourished but unsupplemented women in four countries during the first nine months of lactation. Data from this study 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.



Existing data on the concentrations of water soluble vitamins in human milk are very limited ”

Norbert Sprenger

Human Milk MicroRNAs/Exosomes: Composition and Biological Effects

Bo Lönnerdal (University of California, Davis) examined a newly-discovered component of human milk: the exosomes, extracellular vesicles that contain microRNAs (miRNAs). Their identification opens up an exciting field of research that expands the breadth and depth of the function of human milk.

Exosomes are extracellular vesicles that are produced by a variety of cells including macrophages, lymphocytes, dendritic cells, epithelial and tumor cells. They are present in physiological fluids such as plasma, urine and malignant effusions.

It is well known that exosomes are important in cell-to-cell signaling, but their physiological role *in vivo* is less certain. Isolated milk exosomes have been shown to affect immune responses of peripheral blood mononuclear cells and T regulatory cells. A study on miRNA expression in breast milk uncovered large numbers of miRNAs (281 of 723 known human miRNAs known at the time) and, in particular, high levels of immune-related miRNAs during the first 6 months of lactation. Several of the breast milk miRNAs have been shown to originate from the mammary gland and many are involved in cellular development and immune function.

Another fascinating revelation is exosome-mediated transfer of miRNAs, which presents a novel mechanism for signaling and genetic exchange between cells. One possibility is that exosomes in human milk may survive digestion and deliver miRNAs to the infant's intestinal cells, or, if transferred into the blood stream, to cells in other tissues. Indeed, exosomes and their miRNA cargo can survive proteolytic digestion *in vitro*. Furthermore, these exosomes are taken up by intestinal epithelial cells, where they migrate to the nucleus. These findings highlight the possibility that milk miRNAs may transfer genetic material to the infant and affect gene transcription and regulation of cellular events in various tissues.

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Several of the breast milk miRNAs have been shown to originate from the mammary gland and many of them are involved in cellular development and immune function ”

Bo Lönnerdal

Proteins in Human Milk: Composition and Biological Effects

Sharon Donovan (University of Illinois) reviewed the current evidence on the composition and bioactivity of human milk proteins, with a focus on lactoferrin, osteopontin and the milk fat globule membrane (MFGM).

Human milk contains over 400 proteins that can be broadly classified into 3 categories: caseins, whey proteins and mucins, which are present in the MFGM. Bioactive peptides are formed during digestion of casein and whey, and the glycans from glycoproteins are bifidogenic, adding another dimension of complexity to the functional properties of these molecules.

Lactoferrin is a non-heme iron binding protein that has beneficial effects on iron absorption in the breastfed infant. It also exerts potent bacteriostatic actions. Osteopontin 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 osteopontin supplemented to formula at the concentration present in human milk altered intestinal gene expression in Rhesus monkeys to be more similar to that of breastfed monkeys. Finally, MFGM is the triple membrane system that encapsulates milk fat. It consists of cellular components, including cholesterol, glycerol-phospholipids, sphingolipids and proteins, such as Mucin 1, butyrophilin, CD36, adipophilin, and lactadherin.

The purification of bioactive proteins from bovine milk has paved the way for clinical trials in infants. Data from randomized controlled trials in term and pre-term infants point towards the immune-protective effects of lactoferrin, osteopontin, and the MFGM. Beneficial effects have been seen in terms of reduction in the incidence of diarrhea, respiratory illnesses, fever, and markers of inflammation.

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This new knowledge will facilitate the design of infant formulas that close the gap in health outcomes between breast- and formula-fed infants ”

Sharon Donovan

SESSION 3

Clinical Aspects of Human Milk on Infant Health Outcomes

Chairperson: Sharon Donovan (University of Illinois)



Each of the speakers in Session III highlighted a unique aspect of human milk and its impact on different health outcomes. Many key learnings from this session came from the ingenious methods used to circumvent the methodological challenges associated with the study of infants. Professor Ferdinand Haschke tackled very low birth weight infants and infants from developing countries, identifying the specific nutritional needs of each infant population. Professor Weili Lin used magnetic resonance imaging as a tool for unraveling the complexities of the developing infant brain. Professor Valérie Verhasselt explored the role played by human milk in the maturation of gut immunity and Professor Erika Isolauri explained how the gut microbiome sets the foundation for health and protects against future non-communicable diseases. Professor Paula Meier ended the session with the cost savings attributed to mothers' own milk in very low birthweight infants.

Early Life Nutrition and Growth Trajectories and Metabolic Outcomes

Ferdinand Haschke (Paracelsus Medical University Salzburg and Medical University Vienna) presented data from the follow-up of different infant cohorts that were breastfed according to current recommendations.

The first group consisted of very low birth weight (VLBW) infants. The ESPGHAN recommends an enhanced nutrition strategy for these infants, providing extra nutrients up to 52 gestational weeks. One strategy tested in the clinic was enteral feeding with breast milk that was later fortified with a human milk fortifier, in VLBW infants who were also small for gestational age (SGA) with intrauterine growth restriction (IUGR). The data highlighted two key findings. First, the data support the ESPGHAN enhanced nutrition approach. Second, the data indicate that the same feeding guidelines can be used for VLBW infants who are SGA or IUGR.

Demographic health surveys were used to obtain data on infant growth, nutrition and health in 10 developing countries. Exclusive breastfeeding during the first 6 months of life was associated with a significantly lower prevalence of stunting and wasting. Data from the surveys also revealed that the low quality of complementary foods contributed towards the high stunting rate. Analyses of growth trajectory data indicated that favorable intrauterine growth conditions is key to prevent stunting and wasting during the early years of life.

More clinical data is available for breastfed term infants from developed countries. For this group, maternal obesity is a predictor of overweight/obesity at 5 years of age. Low-protein

follow-up formulas are preferable over high-protein formulas for supporting a normal growth rate that resembles that of breastfed infants. Not surprisingly, high protein intake with milk and complementary foods is also a risk factor for childhood obesity.

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There is no doubt that promotion of breastfeeding in preterm and term newborn infants contributes to optimal growth and health during infancy and childhood, and determines short- and long-term health outcomes ”

Ferdinand Haschke

Early Life Nutrition and Cognitive Development

The first years of life are perhaps the most important for brain maturation and development. Weili Lin (University of North Carolina, Chapel Hill) describes the use of magnetic resonance imaging (MRI) as a tool for studying the effects of nutrition on brain anatomy and function.

A technical challenge with MRI, however, is that it is highly sensitive to movement artifacts. Lin's team has developed several strategies to cope with this problem, allowing the use of MRI to study children of all ages without the use of sedation.

Lin revealed one of the first bodies of quantitative MRI data of early brain structural development in a cohort of normal children up to 6 years of age. This work mapped the changes in the volume of cortical gray matter, the thickness of the cortex, and brain surface area. This information provides an important reference for characterizing brain structural development from birth to early childhood.

Going one step further, Lin's team used resting state functional MRI (rsfMRI) to study the brain's functional maturation process during the first 4 years of life. During the first year, the topologies of the sensorimotor and auditory networks are already highly consistent with those seen in adults. In contrast, higher order brain functional networks are more primitive. The visual networks present a similar topology to those of adults, but undergo tremendous growth in the first year of life.

Together, these structural and functional MRI data provide important quantitative insights into early brain development and provide a key vantage point from which to study the interplay between nutrient intake and early brain development.

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Research on brain imaging, cognitive development, and nutrition intersect in expanded interdisciplinary efforts to understand the gut-brain axis, which could shed further light on our understanding of the complex interaction between brain development and the gut microbiome ”

Weili Lin

Early Life Nutrition and Immune Development

Valérie Verhasselt (University of Western Australia) emphasized the role of breast milk in shaping the composition and function of the gut microbiota, with a focus on gut immunity.

Breast milk contains molecules that interact with gut bacteria, driving their growth and metabolic activity. For example, human milk oligosaccharides (HMOs) not only stimulate the growth of *Bifidobacteria*, but in turn, the bacteria derive short-chain fatty acids (SCFA) from HMOs. *In vivo* studies suggest a role of SCFA in immune modulation, affecting food allergies and gut inflammatory disease. The HMOs in breast milk act as surrogates for the fibers found in solid food, enabling commensal bacteria to produce SCFA in the neonate.

During weaning, factors in breast milk may be important for establishing tolerance towards oral antigens. These factors include TGF β , vitamin A, and IgG. However, not all the antigens present in milk induce tolerance. The presence of the dust mite antigen Der p1 in milk increases the risk of allergy. This highlights the need to identify how maternal milk factors can be modified to counteract the deleterious actions of some antigens.

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. Breast milk also delivers microbiota and nutrients

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Before weaning, the breast milk of mammals provides the neonate with the factors necessary for immune maturation ”

Valérie Verhasselt

to support 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.

Early Life Nutrition and Microbiome Development

Disequilibrium of the gut microbiota has been linked to many clinical conditions, such as inflammatory bowel disease, obesity, allergies and neurodevelopmental disorders. Erika Isolauri (Turku University Hospital) highlighted the importance of breastfeeding as the cornerstone of prevention of non-communicable diseases.

Breastfeeding provides several health benefits that are likely due to promotion of age-appropriate and environment-adjusted gut colonization. There is extensive 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. However, the microbes and other active compounds in breast milk vary greatly according to the mother's health status during pregnancy, and according to the mode of delivery.

How can we exploit our knowledge of human milk composition to modulate long-term health outcomes and reduce disease risk? Before satisfactory preventive measures can be put in practice, important questions remain to be answered. First, we need a better understanding of the mechanisms that underpin the complex immune-mediated and microbiome-associated chronic conditions. Second, long-term follow-up studies are required to determine whether changes in the microbiome underlie the pathogenesis of non-communicable diseases or are merely end results thereof.

Despite these uncertainties, the complex and bidirectional relationship between diet and the gut microbiota is becoming evident. Alterations in the microbiota affect energy acquisition and storage, and may also contribute to the gut immunological milieu. High-energy Western diets alter the microenvironment of the gut leading to perturbation of gut function and to systemic low-grade inflammation. Breastfeeding remains a central element in establishing optimal nutrition and reinforcing long-term immunological outcomes.

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Not only does it provide the infant with nutrients, breast milk also confers immunologic protection at the portal of entry where a major load of antigens is encountered, the gut barrier ”

Erika Isolauri

Human Milk and Clinical Outcomes in Preterm Infants

Paula Meier (Rush University Medical Center) presented the key findings of the LOVE MOM cohort (Longitudinal Outcomes of Very Low Birthweight [VLBW] Infants Exposed to Mothers' Own Milk [MOM]). The goal was to determine the health outcomes and costs of feeding with mothers' own milk in this vulnerable infant population.

The LOVE MOM was a prospective cohort study that enrolled 430 VLBW infants from 2008-2012. In this study, 98% of infants received some MOM, either alone or with formula (no donor human milk was used). The daily 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 independently validated by two neonatologists. Actuarial cost data were calculated and propensity scoring was used to control for the risk of morbidities (confounders).

The study revealed a dose-response relationship between higher amounts of MOM received during neonatal intensive care hospitalization, versus morbidity and costs. Specific outcomes included necrotizing enterocolitis (NEC), late-onset sepsis, bronchopulmonary dysplasia (BPD) and neurodevelopmental parameters. These data suggest that MOM functions over the course of critical exposure periods to

reduce the risk of potentially preventable morbidities and their associated costs in VLBW infants.

The conclusions from this study have several implications for the management of VLBW infants. First, these data support the effectiveness of MOM in reducing the incidence of NEC, sepsis and BPD, all of which are preventable morbidities. Second, MOM is cost-effective as a feeding strategy in neonatal intensive care units. The cost savings derived from MOM feedings are a result of direct reduction in the incidence of neonatal morbidities. Indirect cost savings also stem from a reduction in the incidence of re-hospitalization after discharge. Finally, long-term cost savings are derived from the protective and beneficial health effects of mother's milk over the long run in this vulnerable infant population.

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The primary impact of mother's own milk in this population is the reduction of potentially preventable morbidities ”

Paula Meier

SESSION 4 Research Gaps and Opportunities

Chairperson: J. Bruce German (University of California, Davis)



From metabolomics to synchrotron accelerators, the speakers of Session IV apply new techniques to journey through the universe of human milk and reveal the scope of its influence on infant growth and maturation. Yet the more we know, the less we understand, and we must ever be on the lookout for new clues. Professor Carolyn Slupsky compared the metabolome of breastfed and formula-fed infants in order to clarify the roles of little-known bioactive substances in milk. Professor Katie Hinde explained how milk hormones can shape offspring behavior, by drawing from *in vivo* experimental data. High-throughput analytical and large-scale glycan synthetic methods have uncovered many exciting new factors in human milk. Professor Lars Bode outlined how these new data can be used to understand the function of human milk oligosaccharides. There is also much to be learned from studying other mammals. In describing the development of the tammar wallaby, Professor Kevin Nicholas came closer to understanding the needs of pre-term and low birth weight infants. Professor J. Bruce German ended the session with an overview of the composition and function of the milk fat globule, from its genesis in the mammary epithelium to its digestion in the infant gut.

Metabolomics Approach in Human Milk Research

Although the bulk of research on human milk has focused on proteins, lipids and micronutrients, we now understand that human milk consists of many other factors critical for infant health. Carolyn Slupsky (University of California, Davis) reviewed the state-of-the-art technology that enables an unprecedented study of the impact of human milk.

In the past, most infant feeding studies relied on crude measures of health such as growth or the absence of obvious disease. While these can reveal rudimentary associations between dietary components and general health over the short term, they cannot address the effects of individual nutritional components on specific health outcomes over the long term.

The application of metabolomics techniques based on Nuclear Magnetic Resonance (NMR) and Mass Spectrometry (MS) has enabled the detailed study of different components of human

milk. Comparing the metabolome of breastfed versus formula-fed infants has revealed important clues on how the infant diet affects development. Breastfed infants have lower plasma levels of branched chain amino acids (isoleucine, leucine, and valine) and urea, as well as higher levels of ketone bodies (acetone), acetate, and myo-inositol. Additionally, breastfed infants have lower insulin levels than their formula-fed counterparts 2 hours after feeding. Differences in the levels of these components affect the growth and metabolism of bone, skeletal muscle, the central nervous system, the gastrointestinal tract, blood cells, and other organs.

Further study of human milk and infant metabolism that incorporates the 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 to understand the purpose of specific milk components, individual responses to diet, as well as how diet and genetics combine with the gut microbiota to guide cognitive and metabolic development.



It is now recognized that there are other factors in milk that may be important for infant health, including small molecule metabolites ”

Carolyn Slupsky

Next Generation of Milk's Benefits: Behavioral Outcomes

Katie Hinde (Arizona State University) puts the spotlight on how bioactive hormones in mother's milk shape infant behavior via the hypothalamic-pituitary-adrenal (HPA) axis.

The endocrine pathway of particular interest to behaviorists is the hypothalamic-pituitary-adrenal (HPA) axis, also known as the "stress axis" because it is activated in situations of danger, conflict, or other stresses. Glucocorticoids, produced by the adrenals, play a central role in this context. Glucocorticoids present in the mother's circulation pass into the milk, and are correlated with milk energy density. They are absorbed intact across the infant's intestinal epithelium, and bind to the infant's glucocorticoid receptors. Breastfed infants have a five-fold higher level of glucocorticoid receptors in the intestinal tract compared to formula-fed infants. The density of glucocorticoid receptors in the infant's gut decreases after weaning, suggesting that these receptors are present specifically to receive cortisol signals from mother's milk.

The most comprehensive study of ingested glucocorticoids comes from a series of elegant in vivo experiments. The findings indicate that ingestion of moderately elevated glucocorticoids via mother's milk improves cognition and memory in the offspring, with effects lasting into adulthood. The effects of milk glucocorticoids are partially mediated via glucocorticoid receptors in neural regions such as the

hippocampus and the hypothalamus, particularly in males. In rhesus monkeys, elevated cortisol is associated with better cognitive performance, faster growth, and a more nervous, less confident temperament. Taken together, the data suggest that through cortisol signaling, mothers with fewer somatic resources may be "programming" behaviorally cautious offspring that prioritize growth. In this way, breast milk functions to calibrate infants to their mothers and the broader environmental context.



The term 'lactocrine programming' describes the process by which hormones present in mother's milk permanently shapes physiological processes within the young ”

Katie Hinde

Oligosaccharides: Next Generation Functions

Our knowledge of human milk oligosaccharides (HMOs) has greatly increased due to advances in three key areas: i) high-throughput glycan analysis, ii) large-scale glycan synthesis, and iii) modern microbiome research. Lars Bode (University of California, San Diego) discusses how these novel findings can be extrapolated from bench to baby.

Bode's team identified a specific HMO, disialyllacto-N-tetraose (DSLNT), with the ability to reduce the incidence and severity of necrotizing enterocolitis (NEC) in a rodent model. In parallel to their preclinical work, Bode's team performed a clinical cohort study in 200 mothers and their preterm, very low birth weight infants that were predominantly fed human milk. The findings revealed that infants who developed NEC received less DSLNT with the milk than infants who did not develop NEC. These exciting findings support the validity of the preclinical data. A carefully designed randomized controlled trial is warranted to establish the utility of DSLNT in prevention of NEC.

New HMOs are being identified and added to our molecular repertoire, thanks to advances in glycan synthesis, microbiome research and the associated analytical and bioinformatics tools. Yet our knowledge on the function and potential adverse effects of individual HMOs remains limited. Human milk contains a personalized mixture of a hundred or more different HMOs. Future research must 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 (as they naturally occur in human milk) provide short- or long-term benefits to infants and potentially, to adults.

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Cohesive and consistent results from suitable preclinical *in vitro*, tissue culture and animal models, human cohort associations as well as randomized controlled trials will be required to make conclusive claims about specific HMO functions ”

Lars Bode

Guiding Development - Lessons from Mammalia

Kevin Nicholas (Monash University/University of Melbourne) reveals the lessons learned from the tammar wallaby, and explains how these give new clues towards understanding the function of human milk.

The tammar wallaby is an Australian marsupial with a short, 26-day gestation period and a poorly-developed placenta. At birth, the young are equivalent to a human embryo at mid- to late pregnancy. In the tammar, a portion of the 300-day lactation period may function in a similar manner to the later stages of placental growth in eutherian mammals.

Therefore, examining the timed delivery of bioactive substances in tammar milk may provide clues on the signaling program of the placenta, and other tissues required for normal eutherian development. For example, the lung in newborn marsupials is so immature at birth that the marsupial neonate respirates through the skin for the first 2 weeks. *In vitro* studies have shown that milk collected from marsupials in early lactation (days 20-100), but not late lactation (days 100-300), stimulated proliferation and differentiation of cultured whole lung from mouse embryos.

Another approach has exploited the comparative databases of differentially expressed genes in the tammar mammary gland in early lactation, human milk, colostrum, placenta and the amniote. A focus on genes coding for secreted proteins has facilitated the identification of potential signaling molecules secreted by these tissues. This latter strategy highlighted a number of candidate proteins in the placenta and amniotic fluid,

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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 birth weight babies ”

Kevin Nicholas

and unexpectedly identified signaling molecules in colostrum, prompting the need to re-examine the role of colostrum in the development of both term and preterm babies. These findings underscore the importance of comparative biology as a window into understanding the role of human milk in infant development.

Milk's Structural Dynamics: Nutrient Delivery System

J. Bruce German (University of California, Davis) outlines the journey of the milk fat globule from its assembly in the mammary epithelium to its disassembly in the infant gut, giving new insight into its composition and function.

Milk globules consist of a triglyceride core bound by a phospholipid monolayer, assembled in the endoplasmic reticulum. The globule is later encased in a complete bilayer structure by the mammary epithelial plasma membrane during globule secretion. The size, diversity and composition of milk fat globules change during lactation and as a function of genetics, diet and mammary gland metabolism.

The digestion of these globules in the infant gut is a highly orchestrated process resulting in a complex array of functional by-products. Their ephemeral nature has made it nearly impossible to track these dynamic lipid structures. Recently, high intensity, coherent X-rays in synchrotron accelerators have been used to follow these structures in real time. The latest findings revealed the presence of distinct cubic structures capable of dissolving and transporting both water-soluble and lipid-soluble components in all directions. Thus, the lipid globule is the precursor of a structured delivery system that self-assembles within the infant's intestine.

Although the lipid composition of human milk can vary greatly, some milk fatty acids remain constant across individuals and species. The most abundant single fatty acid in milk is palmitic acid. It is a potent ligand for the PGC1b transcription coactivator in the liver, guiding not only liver lipid secretion but whole body energy metabolism. Palmitoleic acid, a derivative of palmitic acid, is a potent lipokine that controls hepatic gluconeogenesis, muscle lipid uptake, and food intake. Altogether, these findings suggest that many aspects of infant metabolism are under the direct control of the fatty acids in human milk.

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The most complex structure in mammalian milk, the fat globule, has not been effectively examined beyond its simple composition ”

J. Bruce German



CONCLUSIONS

The global initiative to drive breastfeeding for all infants worldwide represents one of the most significant public health interventions. It is therefore critical that any guidance to support breastfeeding is evidence-based. The last decades have seen the emergence of a wealth of data on the composition and function of human milk. This new knowledge is constantly enriching our picture of how human milk sets the foundation for health in later life.

Although it is well accepted that the early years of a child's life are critical for growth and development, we have little mechanistic understanding of how the infant diet shapes short-term and long-term health. One of the key learnings in this workshop is that human milk is not only a source of essential nutrients, but also contains a variety of bioactive substances. These include essential microbes, long-chain fatty acids, complex oligosaccharides, nucleotides, and bioactive signaling proteins and hormones.

We are only just beginning to glimpse at how these components protect against infections, regulate infant development, and modulate long-term outcomes. In the search for answers we must rely not only upon well-designed observational and placebo-controlled clinical studies, but cast our net wide. Clues may be found in diverse places: from sifting through the hundreds of new signals in metabolomics research, to examining unusual species like the tammar wallaby. Modeling studies, genomics, and *in vivo* experiments

will also play a key role in identifying novel substances and testing new concepts. Purification of bioactive substances from human milk will improve infant formula design, narrowing the health gap between breast- and formula-fed infants. A deeper understanding of the function of human milk will also help to enhance outcomes in vulnerable populations, including premature infants, those with low birth weight, and infants from low and middle income countries.

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