Human Milk Composition: Nutrients and Bioactive Factors

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Synopsis

The composition of human milk is the biologic norm for infant nutrition. Human milk also contains many hundreds to thousands of distinct bioactive molecules that protect against infection and inflammation and contribute to immune maturation, organ development, and healthy microbial colonization. Some of these molecules, e.g., lactoferrin, are being investigated as novel therapeutic agents. A dynamic, bioactive fluid, human milk changes in composition from colostrum to late lactation, and varies within feeds, diurnally, and between mothers. Feeding infants with expressed human milk is increasing. Pasteurized donor milk is now commonly provided to high risk infants and most mothers in the U.S. express and freeze their milk at some point in lactation for future infant feedings. Many milk proteins are degraded by heat treatment and freeze-thaw cycles may not have the same bioactivity after undergoing these treatments. This article provides an overview of the composition of human milk, sources of its variation, and its clinical relevance.

Keywords

human milk composition; breastfeeding; infant nutrition; pasteurization; bioactive factors

INTRODUCTION

Exclusive human milk feeding for the first 6 months of life, with continued breastfeeding for 1 to 2 years of life or longer, is recognized as the normative standard for infant feeding.[1,2] Human milk is uniquely suited to the human infant, both in its nutritional composition and in the non-nutritive bioactive factors that promote survival and healthy development.[3] Here, we briefly review the nutritional composition of human milk and provide an overview of its varied bioactive factors, which include cells, anti-infectious and anti-inflammatory agents, growth factors, and prebiotics. Unlike infant formula, which is standardized within a very narrow range of composition, human milk composition is dynamic, and varies within a

feeding, diurnally, over lactation, and between mothers and populations. Influences on compositional differences of human milk include maternal and environmental factors and the expression and management of milk (e.g., its storage and pasteurization). Understanding human milk composition provides an important tool for management of infant feeding, particularly of fragile, high risk infants, and for understanding the potential impact of storage and pasteurization on milk components. Further, we note some bioactive components found in human milk that are being developed and tested for potential medical applications as prophylactic or therapeutic agents.

STAGES OF LACTATION

The first fluid produced by mothers after delivery is colostrum, which is distinct in volume, appearance and composition. Colostrum, produced in low quantities in the first few days postpartum, is rich in immunologic components such as secretory IgA, lactoferrin, leukocytes, as well as developmental factors such as epidermal growth factor.[4-6] Colostrum also contains relatively low concentrations of lactose, indicating its primary functions to be immunologic and trophic rather than nutritional. Levels of sodium, chloride and magnesium are higher and levels of potassium and calcium are lower in colostrum than later milk.[5,6] As tight junction closure occurs in the mammary epithelium, the sodium to potassium ratio declines and lactose concentration increases, indicating secretory activation and the production of transitional milk. The timing of secretory activation (lactogenesis stage II) varies among women, but typically occurs over the first few days postpartum. Delayed onset of lactogenesis is defined as onset >72 hours after delivery and appears to occur more often with preterm delivery and maternal obesity, and may be predicted by markers of metabolic health.[7,8] Biochemical markers in early milk for onset of secretory activation include its sodium content, the sodium to potassium ratio, citrate, and lactose.[9]

Transitional milk shares some of the characteristics of colostrum but represents a period of "ramped up" milk production to support the nutritional and developmental needs of the rapidly growing infant, and typically occurs from 5 days to two weeks postpartum, after which milk is considered largely mature. By four to six weeks postpartum, human milk is considered fully mature. In contrast to the dramatic shift in composition observed in the first month of life, human milk remains relatively similar in composition, although subtle changes in milk composition do occur over the course of lactation.

STUDIES OF HUMAN MILK COMPOSITION

A Medline search using only the phrase "human milk composition" reveals a steady increase in publications since the 1960s, with new components still being identified in human milk, and the functionality of those components under active investigation in many laboratories worldwide. Many studies of human milk composition have been conducted in diverse populations using varied collection, storage and testing methods. The gold standard of milk collection involves sampling of all milk expressed over 24-hours, with collection on multiple occasions from the same individuals over time.[10,11] This method, however, can be costly, limiting the number of participants. Alternatively, studies of milk composition can standardize collection at a specific time of day (e.g., morning) by emptying the entire breast, avoiding collection from a breast that was used for nursing within the past 2-3 hours, with collection on multiple occasions from the same individuals over time.[12] However, most published studies involve non-standardized collection from donors to milk banks whose milk is collected at different times of day, at different timings within a feed or at diverse stages of lactation. Studies of human milk composition also vary in their attention to storage or treatment conditions, such as the number of freeze-thaw cycles, duration of storage, or pasteurization, which may sometimes explain differing study results.

NUTRITIONAL COMPONENTS OF HUMAN MILK

The nutritional components of human milk derive from three sources: Some of the nutrients of milk originate by synthesis in the lactocyte, some are dietary in origin, and some originate from maternal stores. Overall, the nutritional quality of human milk is highly conserved, but attention to maternal diet is important for some vitamins and the fatty acid composition of human milk (see article in this issue by Valentine and Wagner).

Macronutrients

The macronutrient composition of human milk varies within mothers and across lactation but is remarkably conserved across populations despite variations in maternal nutritional status.[13] As shown in Table 1, the mean macronutrient composition of mature, term milk is estimated to be approximately 0.9 to 1.2 g/dL for protein, 3.2 to 3.6 g/dL for fat, and 6.7 to 7.8 g/dL for lactose. Energy estimates range from 65 to 70 kcal/dL, and are highly correlated with the fat content of human milk. Macronutrient composition differs between preterm and term milk, with preterm milk tending to be higher in protein and fat (Table 1). A study in Davis, California examined the association between maternal characteristics and the composition of human milk macronutrients[10] and found that after 4 months postpartum, the macronutrient concentrations of human milk are associated with one or more of the following factors: Maternal body weight for height, protein intake, parity, return of menstruation, and nursing frequency. This study also found that mothers who produce higher quantities of milk tend to have lower milk concentrations of fat and protein but higher concentrations of lactose.

The proteins of human milk are divided into the whey and casein fractions or complexes, with each comprised by a remarkable array of specific proteins and peptides.[14,15] The most abundant proteins are casein, α-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme, and serum albumin.[16,17] Non-protein nitrogen-containing compounds, including urea, uric acid, creatine, creatinine, amino acids, and nucleotides, comprise ~25% of human milk nitrogen. The protein content of milk obtained from mothers who deliver preterm is significantly higher than that of mothers who deliver at term (Table 1). Protein levels decrease in human milk over the first 4 to 6 weeks or more of life regardless of timing of delivery (Figure 1).[11] For feeding preterm infants, the lower level of total protein and specific amino acids from donor (typically, term, late lactation) milk alone is limiting, and requires additional supplementation (see article in this issue by Underwood).[18] Human milk protein concentration is not affected by maternal diet, but increases with maternal body weight for height, and decreases in mothers producing higher amounts of milk.[10]

Human milk fat is characterized by high contents of palmitic and oleic acids, the former heavily concentrated in the 2-position and the latter in the 1- and 3-positions of the triglycerides. Fat is the most highly variable macronutrient of milk. Hindmilk, defined as the last milk of a feed, may contain two to three times the concentration of milk fat found in foremilk, defined as the initial milk of a feed.[19] A study of milk from 71 mothers over a 24-hour period found that the milk fat content was significantly lower in night and morning feedings compared to afternoon or evening feedings.[20] Another study found that ~25% of the variation in lipid concentration between mothers' milk may be explained by maternal protein intake.[10]

The fatty acid profile of human milk varies in relation to maternal diet, particularly, in the long chain polyunsaturated fatty acids (LCPUFAs). LCPUFA intake in the Western world is skewed towards the omega-6 fatty acids, with sub-optimal intake of omega-3 fatty acids. The docosahexanoic acid (DHA) composition of human milk is particularly low in North

American populations; supplementation should be considered for breastfeeding North American women with DHA limited diets.[18,21,22]

The principal sugar of human milk is the disaccharide lactose. The concentration of lactose in human milk is the least variable of the macronutrients, but higher concentrations of lactose are found in the milk of mothers producing higher quantities of milk.[10] The other significant carbohydrates of human milk are the oligosaccharides, which comprise approximately 1 g/dL in human milk, depending on stage of lactation and maternal genetic factors.[23-25] The oligosaccharides are among the non-nutritive bioactive factors discussed below.

The mean values for macronutrients provided here, while valid, belie the observed variability of individual milk samples, particularly in their fat and protein content. Individual donor milk samples from term mothers range at least from 0.6 to 1.4 g/dL for total protein, 1.8 to 8.9 g/dL for fat, 6.4 to 7.6 for lactose, and 50 to 115 kcal/dL for energy.[26] Furthermore, the typical composition of preterm milk differs from that of term milk (Table 1). Thus, nutritional management of high risk infants using human milk requires individualized, adaptive or targeted, strategies for fortification, based on measurement of milk composition and growth monitoring.[27]

Micronutrients

Human milk provides the normative standard for infant nutrition. Nevertheless, many micronutrients vary in human milk depending on maternal diet and body stores (see article in this issue by Valentine and Wagner, 2012), including vitamins A, B1, B2, B6, B12, D, and iodine. The maternal diet is not always optimal, thus, continuing multi-vitamins during lactation is recommended.[28,29] Regardless of maternal diet, Vitamin K is extremely low in human milk and thus, the American Academy of Pediatrics recommends an injection of this vitamin to avoid hemorrhagic disease of the newborn.[28] Vitamin D also occurs in low quantity in human milk, particularly with low maternal exposure to sunshine, a circumstance now common in populations worldwide.[30] While the impact of maternal supplementation with vitamin D on milk composition is under investigation, current pediatric recommendations target postnatal vitamin D supplementation of breastfed infants. Detailed review of the micronutrient composition of human milk is not possible here, but reviews and composition tables are available elsewhere.[28,29,31]

BIOACTIVE COMPONENTS AND THEIR SOURCES

Bioactive components of food are defined as elements that "affect biological processes or substrates and hence have an impact on body function or condition and ultimately health".[32] Bioactive components in human milk come from a variety of sources; some are produced and secreted by the mammary epithelium, some are produced by cells carried within the milk,[33] while others are drawn from maternal serum and carried across the mammary epithelium by receptor-mediated transport. Further, the secretion of the milk fat globule (MFG) into milk by the mammary epithelium carries with it a diverse collection of membrane-bound proteins and lipids into the milk.[34] Together these methods produce the variety of bioactive components in human milk. For example, in lactating women, antigen-specific B cells home to the mammary gland, where polymeric immunoglobulin receptors (pIgR) transport sIgA into the lumen of the duct.[35] An alternative example is vascular endothelial growth factor (VEGF), which is found at concentrations significantly higher in milk than maternal serum, indicating a mammary gland source.[36,37] Understanding the sources of bioactive components of milk also helps to explain the variability in milk concentrations that are observed following maternal use of specific medications (see article in this issue by Rowe, Baker and Hale).

What are the clinical implications of research on human milk bioactive factors? The depth of scientific evidence is such that in patient or public education, it is valid to clarify that human milk is not "merely nutrition." Rather, human milk contains a variety of factors with medicinal qualities that have a profound role in infant survival and health. Thus, safe donor milk substitutes are needed for infants at medical risk when mother's own milk is not available. Proteomic analysis has discovered thematic distinctions in the proteins that compose milk at differing stages of lactation, as well as differences between term and preterm milks.[14,15] These studies suggest that when donor milk is needed, it should be matched to the developmental stage of the infant whenever feasible, although this is often difficult in practice. Furthermore, recognition of potent, bioactive human milk factors indicates the importance of preserving their biologic activity, to the extent possible, through the process of milk collection, storage, and pasteurization. Finally, recognition of the unique mechanisms by which human milk protects and enhances development provides models for new preventive and therapeutic approaches in medicine.

A complete characterization of bioactive factors of human milk is beyond the scope of this review. Here, we focus on a selected set of bioactive factors that vary between mothers of term and preterm infants, or over the course of lactation, and thus represent responsiveness to the changing needs of the infant (see Table 2). Many of these factors act synergistically, such that consumption of human milk is superior to supplementation with individual factors or their combinations.[38]

GROWTH FACTORS

Human milk contains numerous growth factors that have wide-ranging effects on the intestinal tract, vasculature, nervous system, and endocrine system.

Intestinal maturation, and repair: Epidermal growth factor (EGF)

Found in both amniotic fluid and breast milk,[38-40] EGF is critical to the maturation and healing of the intestinal mucosa. EGF is resistant to low pH and digestive enzymes, allowing it to pass through the stomach to the intestine, where it stimulates the enterocyte to increase DNA synthesis, cell division, absorbance of water and glucose, and protein synthesis.[41,42] There are multiple protective mechanisms of action for EGF in the infant intestine, including inhibition of programmed cell death, and correcting alterations in intestinal and liver tight junction proteins induced by pro-inflammatory TNF-α.[43] Heparin-binding growth factor (HB-EGF) is a member of the EGF family, and the primary growth factor responsible for damage resolution following hypoxia, ischemia-reperfusion injury, hemorrhagic shock/ resuscitation injury, and necrotizing enterocolitis.[44] EGF is highest in early milk and decreases over lactation.[45,46] The average EGF level in colostrum is 2000-fold higher and in mature milk is 100-fold higher than in maternal serum.[41] Further, preterm milk contains higher levels of EGF than term milk.[45,46]

Growth and development of the enteral nervous system: Neuronal growth factors

The immaturity of the newborn intestine extends to the enteral nervous system, which requires brain-derived neurotrophic factor (BDNF) and glial cell-line derived neurotrophic factor (GDNF) for its development.[47] BDNF can enhance peristalsis, a function which is frequently impaired in the pre-term gut.[48] Rodents who lack GDNF display a profound loss of neurons in the ENS.[49] BDNF, GDNF, and a related protein, ciliary neurotophic factor (CNTF), are detected in human milk up to 90 days after birth.[50,51] In human cells, breast milk-derived GDNF increases neuron survival and outgrowth.[51]

Tissue growth: The insulin-like growth factor (IGF) superfamily

IGF-I and IGF-II, as well as IGF binding proteins and IGF-specific proteases, are found in human milk.[52-54] Levels are highest in colostrum, and steadily decline over the course of lactation.[55,56] There are no significant differences between preterm and term milk, with the exception of IGF binding protein-2, which is higher in preterm milk.[52,57] In rodents, administration of human IGF-I during surgical stress/total parental nutrition causes increased tissue growth,[58] and attenuates intestinal atrophy,[59] though no human trial has been conducted.[60] IGF-1 may also play a role in the survival of enterocytes following intestinal damage from oxidative stress.[57] Breastfed infants have higher circulating IGF-I in the serum.[61] IGF can be taken up in a bioactive form by intestines, and transported into the blood.[54,62] The function of absorbed IGF has not been fully detailed, but enteral administration of physiological levels of IGF-I stimulates erythropoiesis and increases hematocrit.[63]

Regulation of the vascular system: Vascular endothelial growth factor (VEGF)

Angiogenesis is regulated primarily by the relative expression of VEGF and its antagonists. VEGF concentration is highest in colostrum in both preterm and term human milk, with preterm milk containing less VEGF than term milk.[64] In retinopathy of prematurity (ROP), it is thought that pulmonary immaturity, supplemental oxygen, and negative regulation of VEGF lead to dysregulated vascularization of the retina,[65,66] suggesting a mechanism by which human milk may help reduce the burden of ROP.

Intestinal development and prevention of anemia: Erythropoietin (Epo)

Milk contains significant quantities of Epo, which is the primary hormone responsible for increasing red blood cells (RBC). Blood loss, intestinal pathology, and immaturity of the hematopoietic system all contribute to anemia of prematurity, which profoundly impacts growth and development,[67] thus, some suggest that Epo may help prevent anemia of prematurity,[68] but administration of Epo has shown mixed results.[69] Administration of Epo in conjunction with iron, however, may increase hemoglobin and hematocrit levels.[70] A small trial of enteral Epo in preterm infants showed increased serum and reticulocyte levels.[71] In addition, Epo is an important trophic factor and tightens intestinal junctions.[72] There is some evidence that Epo may help protect against mother-to-child transmission of HIV,[73] and may help reduce risk of necrotizing enterocolitis.[72,74]

Growth-regulating hormones: Calcitonin and somatostatin

Calcitonin and its precursor procalcitonin are present in large quantities in milk.[75] Enteric neurons express the calcitonin receptor-immunoreactivity (CTR-ir) from late gestation into infancy.[76] Somatostatin is rapidly degraded in the jejunum and not transferred through the intestinal wall, but delivery with milk protects it from degradation and maintains bioactivity within the lumen.[77] Somatostatin normally inhibits growth factors, but its role in human milk remains unclear.[78]

Regulating metabolism and body composition: Adiponectin and other hormones

Adiponectin is a large, multi-functional hormone that actively regulates metabolism and suppresses inflammation. Found in large quantities in human milk, adiponectin can cross the intestinal barrier, and appears to modify infant metabolism.[79,80] Levels of adiponectin in milk correlate inversely with infant weight and BMI while exclusively breastfeeding, thus, some have proposed that adiponectin in human milk may contribute to reduced incidence of overweight and obesity in later life, though this remains to be determined.[79,81] Other metabolism-regulating hormones found in effective quantities in human milk are leptin,

resistin, and ghrelin, which appear to play an important role in regulating energy conversion, body composition, and appetite control.[82-85]

IMMUNOLOGICAL FACTORS

Feeding human milk protects against infection and inflammation,[86] and early milk is enriched in immune factors that help to ensure infant survival.[15] The specific protective components of human milk are so numerous and multi-functional, that science is just beginning to understand their functions.

Transfer of living protection and programming: Cells of human milk

Human milk contains a variety of cells, including macrophages, T cells, stem cells, and lymphocytes.[87-93] In early lactation, the breastfed infant may consume as many as 10^{10} maternal leukocytes per day. The relative quantity of these cells differs among mothers and is reported to differ in the milk of infants who develop allergy.[87] About 80% of the cells in early milk are breast milk macrophages, which originate as peripheral blood monocytes that exit the bloodstream and migrate into milk through the mammary epithelium. Phagocytosis of human milk components transforms these monocytes into potent breast milk macrophages with unique functional features, including the ability to differentiate into dendritic cells that stimulate infant T-cell activity.[89,93] This capability provides broadly powerful protection against pathogens while stimulating development of the infant's own immune system. In women infected with HIV-1 and HTLV-1, however, the activity of these cells unfortunately enables mother to infant viral transmission (see article by Lawrence in this issue). Stem cells have also been identified in human milk;[88,90] their function is under investigation.

Communication between cells: Cytokines and chemokines

Cytokines are multi-functional peptides that act in autocrine/paracrine fashion.[33] Chemokines are a special class of chemotactic cytokines that induce movement of other cells. Human milk cytokines can cross the intestinal barrier, where they "communicate" with cells to influence immune activity. While many cytokines and chemokines have multiple functions, milk-borne cytokines may be grouped broadly into those that enhance inflammation or defend against infection, and those that reduce inflammation. A complete review of the cytokines of human milk is available elsewhere,[33,94-96]

The TGF-β family constitutes the most abundant cytokines of human milk, and consists of three isoforms, of which TGF-β2 predominates.[97-99] Milk-borne TGF-β regulates inflammation and wound repair, and helps prevent allergic diseases. TGF-β is converted to its active form by the low pH of the stomach.[100] Only 15 minutes after TGF-β1-deficient mouse pups are suckled by heterozygous dams, TGF-β1 is measurable in the pup's serum and tissue.[101] Milk TGF-β has tolerance-enhancing activity in the intestinal tract,[100,102-104] a proposed mechanism for its anti-allergenic effects.

Granulocyte-colony stimulating factor (G-CSF), identified in human milk decades ago,[105] has beneficial effects on intestinal development and the treatment of sepsis. While milk-borne G-CSF survives transit through the stomach, it is not appreciably absorbed and acts at the intestinal surface ,[106,107] where it increases villi, crypt depth, and cell proliferation.[108] Other regulatory cytokines found in milk are IL-10 and IL-7;[109] milk-borne IL-7 is known to cross the intestinal wall and influence thymic development.[110]

Pro-inflammatory cytokines TNF-α, IL-6, IL-8, and IFNγ are also found in mother's milk, generally at lower levels, and decreasing over lactation.[4,111] The levels of these cytokines in milk are also associated with the timing of delivery.[4,111] Intriguingly, soluble TNF Receptors I and II, which neutralize TNFα, also are found in human milk.,[112] thus, most

TNFα may be bound and not freely active in milk.[113] Both IL-8 and TNFα are modestly increased in mature milk of mothers who had preeclampsia.[114] Levels of TNFα correlate positively with other inflammatory cytokines.[115] Interleukin-6 (IL-6) is associated with systemic inflammation and fever.[115] The role of the inflammatory cytokines found in human milk remains under investigation, but they are known to be engaged in the recruitment of neutrophils, enhance intestinal development, and IL-8 may help protect against TNFα-mediated damage.[116][117,118] Higher levels of IL-6 and IL-8 occur in mastitis, with higher cytokine levels confined to only the affected lobes.[119,120] Interferon gamma (IFNγ) has also been widely studied due to its pro-inflammatory role; IFNγ enhances the Th1/inflammation response while suppressing the Th2/allergic response.[94] The colostrum of allergic mothers contains lower IFNγ but higher Th2 cytokines IL-4 and IL-13 compared to non-allergic mothers.[121]

Protection from infection: Acquired and innate factors

A recent study in Nepal found a 9-fold increase in risk of diarrhea in children who were not breastfed,[122] consistent with previous global studies.[123] Infants are born with immature acquired immunity, and rely on maternal antibodies for defense against pathogens.[124] Human milk sIgA-antigen complexes are taken up and processed by intestinal dendritic cells, which allows for antigen recognition while maintaining a non-inflammatory environment.[125] While sIgA is the predominant antibody of human milk, milk also contains IgM and IgG, the latter becoming more abundant in later lactation.[15] A comprehensive review of human milk immunoglobulins is available elsewhere.[124,126,127]

A set of innate, multi-functional molecules also provide significant protection against infection. These molecules are typically highest in colostrums, and decrease over lactation. Among the most abundant of these is lactoferrin, an iron-binding glycoprotein belonging to the transferrin family,[128][129] which is effective against many different bacteria, viruses, and fungi.[130-134] Another glycoprotein, lactadherin, was initially identified in the milk fat globule.[135] Milk-borne lactadherin survives transit through the stomach,[136] and prevents rotaviral infection in the newborn.[137] Following infection or damage, lactadherin mediates phagocytic uptake of apoptotic cells[138,139] and stimulates a signaling cascade that results in decreased inflammation via blockade of TLR4 and NF-κB signaling.[138,140] Lactadherin promotes healing during intestinal inflammation[140,141] and a tolerogenic phenotype in intestinal dendritic cells and macrophages,[138,142] which is important to maintaining gut health. Another multi-functional protein, bile salt stimulating lipase (BSSL)[143] is a highly-glycosylated enzyme that breaks down milk fats, thereby releasing their energy for infant metabolism. Milk-borne BSSL also protects infants from viral infection, including Norwalk and HIV. BSSL binds to dendritic cells, preventing HIV trans-infection of CD4+ T cells.[144,145] The bioactive region of BSSL is modified by the glycans present, which change with maternal genetics and over lactation.[143,146]

The milk fat globule (MFG) contains mucins (MUC1, MUC4, and potentially others) derived from the maternal plasma membrane.[147] These mucins are multi-functional, but most importantly, protect infants from infection. For example, MUC1 blocks infection by HIV and rotavirus[148,149] and both MUC1 and MUC4 block infection by *Salmonella enterica* serovar *typhimurium* and Norwalk virus.[146,150,151]

Selection for the growth of beneficial organisms: Oligosaccharides

The human milk oligosaccharides (HMOS) range from 3 to 32 sugars in size, and differ in composition from those of any other mammal.[23,24] Though non-nutritive to the infant, HMOS constitute a remarkable quantity of human milk, similar to the quantity of total protein. These structures are synthesized glycosyltransferases, enzymes that also synthesize

similar structures in other human secretions and on mucosal surfaces. The HMOS are "prebiotic" agents that selectively encourage the growth of beneficial (probiotic) organisms. In addition, the HMOS and their protein conjugates are recognized as pathogen-binding inhibitors that function as soluble "decoy" receptors for pathogens that have an affinity for binding to oligosaccharide receptors expressed on the infant's intestinal surface. Mothers vary in the specific structures of HMOS in their milk as a result of genetic differences similar to blood group types.[23-25] This variation in HMOS composition, unlike blood group types, does not create incompatability, so that all mothers may be considered "universal donors." Rather, the variation in HMOS composition among mothers is thought to promote human survival as pathogens differ in their affinity for binding to specific oligosaccharides. Protection by some forms of HMOS but not others has been shown in relation to diarrhea caused by specific pathogens[23,24] and HIV.[152] The apparent differences in lactose and HMOS composition of preterm milk requires further investigation.[25,153]

It was long believed that human milk was sterile, but it is now recognized that human milk harbors a microbial community, the composition of which appears to change with maternal characteristics and over the course of lactation.[154,155] The HMOS influence intestinal colonization and may also influence the bacterial community composition of milk.

IMPACT OF STORAGE AND PASTEURIZATION ON MILK COMPONENTS

Increasingly, feeding human milk occurs apart from feeding at the mother's breast. Most lactating women in North America now express and store their milk at some time.[156] Varying degrees of nutrient loss occur depending on the nutrient and the storage methods. For vitamin C, loss occurs rapidly, even during the process of feeding freshly expressed human milk by bottle.[155] For multiple human milk components, however, significant degradation may only occur with long term storage and freeze-thaw cycles, which tend to reduce bactericidal capacity. The Academy of Breastfeeding Medicine has a protocol for home storage of human milk that can be used to guide mothers in these activities to optimize the integrity of expressed and stored milk (see article in this issue by Chantry and Howard, ABM Protocol #8).

Another major movement has been the development of milk sharing via donor human milk banks or alternatively, via internet milk sharing (see article in this issue by Landers and Hartmann, 2012). Due to the known or theoretical risk of transmitting pathogens via human milk, a variety of protocols have been developed for pasteurization of donor milk, including: High temperature short time (HTST) heating (72°C for 15 seconds); Holder pasteurization (62.5°C for 30 minutes, a low temperature long time [LTLT] method); and flash heating (a low-tech HTST method that involves heating a jar of milk in a water bath which is rapidly brought to a rolling boil, then milk is removed and rapidly cooled). Flash heating was developed with the goal of achieving effective pasteurization in a home environment in resource-poor areas of the world to prevent transmission of HIV.

Each pasteurization method has been investigated for its ability to eliminate pathogens while preserving as many bioactive components and nutrients as possible.[157-159] Unfortunately, heat treatment of human milk reduces the concentration and functionality of its bioactive components, particularly in protein composition and function. Significant reductions have been demonstrated after pasteurization in sIgA, lysozyme, BSSL, cytokines, lipases, TGF-β, and adiponectin, among other proteins.[160-162] Pasteurization damages some proteins more than others,[162] but is particularly damaging when paired with the multiple freeze-thaw cycles that can occur with donated milk.[163] The degree of impact appears to vary by pasteurization method. Boiling is likely the most damaging, while Holder pasteurization, the method utilized by the milk banks associated with the Human Milk Banking Association of

North America, may be more destructive than HTST (see also in this issue, Landers & Hartmann). Though a low-tech method, flash-heat treatment appears to preserve the bacteriostatic activity of human milk.[158] Much work remains to be done regarding the bioactivity of human milk components following milk treatment.[162]

HUMAN MILK COMPONENTS IN RELATION TO INFANT HEALTH

Management or augmentation of human milk factors

Recognition of the dynamic variability of human milk is important to the management of human milk feeding. For example, mothers of term infants are commonly advised to empty an entire breast prior to feeding from the other breast. As hindmilk is more energy dense due to its higher lipid content, this recommendation ensures that infant satiety and energy needs for growth are well met. Hindmilk has been successfully used to improve growth of very premature infants, and is recommended for their nutritional management.[164] Furthermore, understanding the dynamic variability of human milk, donor milk banks have established protocols for "pooling" different milk donations to achieve a more uniform milk for distribution.

Some milk factors can be modified through dietary intake or exposures in order to optimize infant growth and health. For example, maternal diet influences the DHA content of milk, which is below recommended levels in many populations. Valentine et al have shown that maternal supplementation with 1 g of preformed DHA daily significantly increases milk DHA levels, which thereby improves the dietary DHA of breastfed infants.[164] Another approach to modification of human milk is through maternal immunization, which is being actively pursued as a public health strategy. Trials of maternal immunization have demonstrated significant increases in the level of protective immunoglobulins in milk and dramatically reduced influenza in mothers and infants.[165]

Potential novel therapeutics based on human milk components

The important maternal act of breastfeeding provides many bioactive components to infants. Some of these components have been proposed as novel medical agents for prevention or treatment of disease, including lactoferrin, lactadherin, epidermal growth factor, erythropoietin, and human milk oligosaccharides. While various human milk components have demonstrated significant biologic activity *in vitro* or *in vivo*, only a few have been tested in clinical trials to improve specific health outcomes. One of the most studied of these factors is lactoferrin. Testing lactoferrin as a novel medical agent has been advanced by the use of recombinant lactoferrin or bovine lactoferrin, due to its high homology with human lactoferrin. An Italian study of pre-term infants found that supplementation with bovine lactoferrin, alone or in combination with *Lactobacillus casei* sps. *rhamnosus* (LGG), significantly reduced the risk of late-onset sepsis.[133,134] A current phase 1/2 trail is evaluating the safety and efficacy of an oral lactoferrin solution to prevent infection in preterm infants.[161] Many other individual milk components may be tested in future for specific applications, but human milk remains the unique and potent standard for feeding infants.

CONCLUSION

Human milk is a dynamic, multi-faceted fluid containing nutrients and bioactive factors needed for infant health and development. Its composition varies by stage of lactation and between term and preterm infants. While many studies of human milk composition have been conducted, components of human milk are still being identified. Standardized, multi-population studies of human milk composition are sorely needed to create a rigorous, comprehensive reference inclusive of nutrients and bioactive factors. Nevertheless,

knowledge of human milk composition is increasing, leading to greater understanding of the role of human milk in infant health and development.[166]

Acknowledgments

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Key points

- Human milk composition provides the standard for human infant nutrition, including the bioactive components that safeguard infant growth and development.

- The composition of human milk is variable within feeds, diurnally, over lactation, and between mothers and populations. This variability has benefits for infant health and survival, but for high risk infants requiring close nutritional oversight, supplementation, and strategies for managing the variability of human milk feeds are needed.

- The composition of human milk can be altered with treatment of expressed milk, including its storage and pasteurization. Thus, attention to management of expressed milk is important.
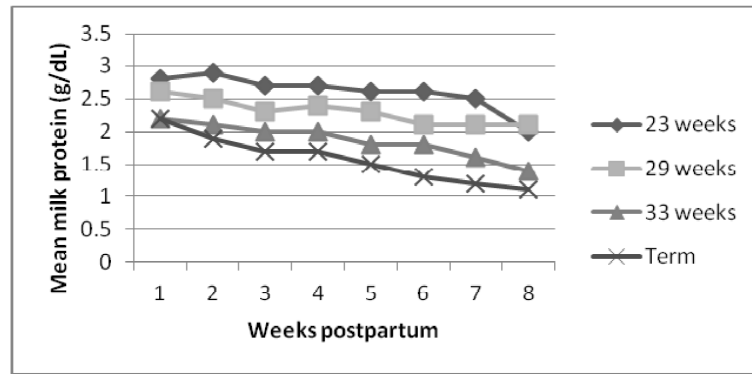
Figure 1.
Milk protein concentrations, comparing milk from mothers who delivered preterm and term, by gestational age at delivery and weeks postpartum (adapted from Bauer & Gerss, 2011).

Table 1

Macronutrient (g/dL) and energy (kcal/dL) composition of human milk from specified references

Author (year), n	Protein Mean (± 2 SD)	Fat Mean (± 2 SD)	Lactose Mean (± 2 SD)	Energy Mean (± 2 SD)
Term infants, 24-hour collection, mature milk				
Nommsen et al (1991), n=58	1.2 (0.9, 1.5)	3.6 (2.2, 5.0)	7.4 (7.2, 7.7)	70 (57, 83)
Donor human milk samples				
Wojcik et al (2009), n=415	1.2 (0.7, 1.7)	3.2 (1.2, 5.2)	7.8 (6.0, 9.6)	65 (43, 87)
Michaelsen et al (1990), n=2553	[a] 0.9 (0.6, 1.4)	[a] 3.6 (1.8, 8.9)	[a] 7.2 (6.4, 7.6)	[a] 67 (50,115)
Representative values of mature milk, term infants				
Reference standard	0.9	3.5	6.7	65 to 70
Preterm, 24-hour collection, first 8 weeks of life				
Bauer & Gerss (2011) Born <29 weeks, n=52 Born 32-33 weeks, n=20	 2.2 (1.3, 3.3) 1.9 (1.3, 2.5)	 4.4 (2.6, 6.2) 4.8 (2.8, 6.8)	 7.6 (6.4, 8.8) 7.5 (6.5, 8.5)	 78 (61, 94) 77 (64, 89)
Preterm donor milk				
Hartmann (2012), n=47	1.4 (0.8, 1.9)	4.2 (2.4, 5.9)	6.7 (5.5, 7.9)	70 (53, 87)

[a] Median (lower bound)

Nommsen et al, 1991: Davis, CA mothers at 3 months postpartum

Wojcik et al, 2009, U.S. milk bank donors

Michaelsen et al, 1990, Danish milk bank donors

Reference standard based on expert review: Pediatric Nutrition Handbook, 6th edition, Table C-1, p. 1201 (Mature Milk)

Bauer & Gerss, 2011, preterm infants 23 to 33 weeks gestational age

Hartmann (2012), Australian donor mothers of preterm infants (see article this issue by Landers and Hartmann)

Table 2

Major Bioactive Factors in Human Milk

Component	Function	Reference
Cells		
Macrophages	Protection against infection, T-cell activation	Jarvinen, 2002, Yagi, 2010, Ichikawa, 2003
Stem cells	Regeneration and repair	Indumathi, 2012
Immunoglobulins		
IgA/sIgA	Pathogen binding inhibition	Van de Perre, 2003, Cianga, 1999; Brandtzaeg, 2010; Kadaoui, 2007; Corthësy, 2009; Hurley, 2011; Agarwal, 2010; Castellote, 2011
IgG	Anti-microbial, activation of phagocytosis (IgG1, IgG2, IgG3); anti-inflammatory, response to allergens (IgG4)	Cianga, 1999; Agarwal, 2010
IgM	Agglutination, complement activation	Brandtzaeg, 2010; Van de Perre, 1993; Agarwal, 2010
Cytokines		
IL-6	Stimulation of the acute phase response, B cell activation, pro-inflammatory	Ustundag, 2005; Meki, 2003; Mizuno, 2012; Agarwal, 2010; Castellote, 2011
IL-7	Increased thymic size and output	Aspinall, 2011; Ngom, 2004
IL-8	Recruitment of neutrophils, pro-inflammatory	Claud, 2003; Ustundag, 2005; Meki, 2003; Maheshwari, 2002; Maheshwari, 2003; Maheshwari, 2004; Hunt, 2012; Agarwal, 2010; Castellote, 2011; Mehta, 2011
IL-10	Repressing Th1-type inflammation, induction of antibody production, facilitation of tolerance	Meki, 2003; Agarwal, 2010; Castellote, 2011; Mehta, 2011
IFNγ	Pro-inflammatory, stimulates Th1 response	Hrdý, 2012; Agarwal, 2010
TGFβ	Anti-inflammatory, stimulation of T cell phenotype switch	Penttila, 2010; Kalliomäki, 1999; Saito, 1993; Nakamura, 2009; Letterio, 1994; Ando, 2007; Ozawa, 2009; Donnet-Hughes, 2000; Verhasselt, 2008; Verhasselt, 2010; Penttila, 2003; Mosconi, 2010; Okamoto, 2005; Penttila, 2006; Peroni, 2009; McPherson, 2001; Ewaschuk, 2011; Castellote, 2011
TNFα	Stimulates inflammatory immune activation	Rudloff, 1992; Ustundag, 2005; Erba ci, 2005; Meki, 2003; Agarwal, 2010; Castellote, 2011
Chemokines		
G-CSF	Trophic factor in intestines	Gilmore, 1994; Gersting, 2003; Calhoun, 2003; Gersting, 2004;
MIF	Macrophage Migratory Inhibitory Factor: Prevents macrophage movement, increases anti-pathogen activity of macrophages	Magi, 2002; Vigh, 2011
Cytokine Inhibitors		
TNFRI and II	Inhibition of TNFα, anti-inflammatory	Buescher, 1998; Buescher, 1996; Meki, 2003; Castellote, 2011
Growth Factors		
EGF	Stimulation of cell proliferation and maturation	Patki, 2012; Kobata, 2008; Hirai, 2002; Wagner, 2008; Dvorak, 2003; Dvorak, 2004; Chang, 2002; Khailova, 2009; Coursodon, 2012; Clark, 2004; Castellote, 2011; Untalan, 2009
HB-EGF	Protective against damage from hypoxia and ischemia	Radulescu, 2011
VEGF	Promotion of angiogenesis and tissue repair	Loui, 2012; Ozgurtas, 2011

Component	Function	Reference
NGF	Promotion of neuron growth and maturation	Rodrigues, 2011; Boesmans 2008; Sánchez 1996; Fichter, 2011;
IGF	Stimulation of growth and development, increased RBCs and hemoglobin	Chellakooty, 2006; Blum, 2002; Burrin 1997; Philipps, 2002; Milsom, 2008; Prosser, 1996; Elmlinger, 2007; Peterson, 2000; Murali, 2005; Corpeleijn, 2008; Baregamian, 2006; Baregamian, 2012; Büyükkayhan, 2003; Philipps, 2000; Kling, 2006;
Erythropoietin	Erythropoiesis, intestinal development	Carbonell-Estrany 2000; Juul, 2003; Kling, 2008; Miller-Gilbert, 2001; Pasha, 2008; Soubasi, 1995; Shiou, 2011; Arsenault, 2010; Miller, 2002; Untalan, 2009
Hormones		
Calcitonin	Development of enteric neurons	Struck, 2002; Wookey, 2012
Somatostatin	Regulation of gastric epithelial growth	Chen, 1999; Rao, 1999; Gama, 1996
Anti-microbial		
Lactoferrin	Acute phase protein, chelates iron, anti-bacterial, anti-oxidant	Adamkin, 2012; Sherman, 2004; Manzoni, 2009; Hirotani, 2008; Buccigrossi, 2007; Velona, 1999
Lactadherin/ MFG E8	Anti-viral, prevents inflammation by enhancing phagocytosis of apoptotic cells	Stubbs, 1990; Kusunoki, 2012; Aziz, 2011; Shi, 2004; Chogle, 2011; Baghdadi, 2012; Peterson, 1998; Newburg, 1998; Shah, 2012; Miksa, 2006, Komura, 2009; Miksa, 2009; Wu, 2012; Matsuda, 2011; Silvestre, 2005
Metabolic hormones		
Adiponectin	Reduction of infant BMI and weight, anti-inflammatory	Martin, 2006; Newburg, 2010; Woo, 2009; Woo, 2012; Ley, 2011; Dundar 2010; Ozarda, 2012; Savino, 2008; Weyerman, 2006
Leptin	Regulation of energy conversion and infant BMI, appetite regulation	Savino, 2008; Savino, 2012a; Savino 2012b; Palou, 2009; Weyermann, 2006
Ghrelin	Regulation of energy conversion and infant BMI	Savino, 2008; Savino, 2012; Dundar 2010
Oligosaccharides & glycans		
HMOS	Prebiotic, stimulating beneficial colonization and reducing colonization with pathogens; reduced inflammation	Newburg, 2005; Morrow, 2005; DeLeoz, 2012; Marcoba, 2012; Kunz, 2012; Ruhaak, 2012; Bode, 2012
Gangliosides	Brain development; anti-infectious	Wang B, 2012
Glycosaminoglycans	Anti-infectious	Coppa, 2012; Coppa 2011
Mucins		
MUC1	Block infection by viruses and bacteria	Ruvoen-Clouet, 2006; Liu, 2012; Sando, 2009; Saeland, 2009; Yolken, 1992
MUC4	Block infection by viruses and bacteria	Ruvoen-Clouet, 2006; Liu, 2012; Chaturvedi, 2008