Art and Science of Breastfeeding & Beyond

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Foreword **VD Patil**



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Composition of Human Milk

Vijay Venkataiah, K Nagaraj, Durgappa H

"If a new vaccine became available that could prevent one million or more child deaths a year, and that was moreover cheap, safe, administered orally, and required no cold chain, it would become an immediate public health imperative. Breastfeeding can do all of this".

-Lancet, A Warm Chain For Breastfeeding

INTRODUCTION

Breastmilk is an ideal infant food and is the complete source of nutrition up to 6 months of age and continues to contribute major nutrients and calories throughout the duration of breastfeeding. Breast milk is a "dynamic living fluid that is baby-specific, species-specific, and tailor-made".¹ The quantity of breast milk secretion varies among mothers with the time of the day and frequency of emptying of the breast.

BREAST MILK SECRETION

The first milk, colostrum, secreted during the first 5 days of delivery (40–50 mL/day), which is rich in nutrients and bioactive factors, plays an important role in immune functions (*natural vaccine*) and is critical for the survival of infants (*liquid gold*). Thereafter, the amount of breast milk secretion during the first 6 months is 600–800 mL/day, between 6 and 12 months is 400–600 mL/day, and in the second year of life is around 400 mL/day. However, the amount of breast milk secretion will be influenced by the efficiency and frequency of breast milk removal.²

VARIATIONS IN THE COMPOSITION OF BREAST MILK Colostrum

Colostrum is thick, yellowish milk produced during the first few days of childbirth, which is nutrient-dense, rich in bioactive factors, and critical for providing immunity to infants. It has a high amount of immune cells (lymphocytes), antibodies [immunoglobulin A (IgA), IgG, and IgM], antimicrobial agents, (lactoferrin, lysozyme, and lactoperoxidase) various complements, and proline-rich polypeptides. The concentration of human milk oligosaccharides (HMOs) is highest in colostrum (20-25 g/L) and the wheycasein ratio in the colostrum is 90:10. whereas in mature milk, it is 60:40. Several immunomodulators and growth factors are found in higher concentration in colostrum. All these bioactive components in colostrum provide passive immunity to the newborn. Colostrum also acts as a mild *laxative* that enhances the elimination of the bilirubinrich meconium from the gut, thus favoring the newborn's digestive system to grow and function properly.³

The breast milk which is secreted between 7 days and 2 weeks of the postpartum period is called *transitional milk* and the milk that is produced beyond 2 weeks of the postpartum period is called *mature milk*. The breast milk secreted at the beginning of feeding is called *foremilk*, which is bluish, watery, and rich in proteins and satisfies the thirst of an infant. The breast milk secreted toward the end of breastfeeding is called *hind milk*, which is yellowish, thick, and rich in fat, satisfying the hunger of an infant.⁴

Preterm Milk

Preterm milk is the milk secreted in the mother who had preterm labor that furnishes all the necessary nutrients for the preterm baby, thus tailored for preterm babies. It contains a higher concentration of protein, free amino acids, and lipids compared to term milk. Calcium is lower in preterm milk, while copper and zinc are in higher concentration. Lactose is relatively low in preterm milk and glycosaminoglycans (GAGs—antiadhesive agents against harmful microbes on the infants' intestinal wall) are in higher concentration compared to term milk.¹⁵

Human milk contains macronutrients, micronutrients, minerals, several bioactive factors, and genetic materials.

Macronutrients in Human Milk

Macronutrients in human milk; proteins, carbohydrates, and fats.

- *Protein components in human milk:* They include whey proteins, caseins, and mucins.
 - Whey proteins: They include lactalbumin, lactoferrin, secretory IgA, lysozyme, haptocorrin, and amylase which are water soluble. Generally, these whey proteins are

responsible for the development of the immune system. Alpha-lactalbumin comprises 10-20% of the total protein in human milk. It provides all essential amino acids to the infant and increases the absorption of calcium, zinc, and iron. It also has antimicrobial activity.⁶ Lactoferrin is a glycoprotein that facilitates the uptake of iron in enterocytes by binding with two ferric ions present in breast milk. Lactoferrin binds to an iron molecule (iron chelation), and thus making iron unavailable to irondependent microorganisms, namely Escherichia coli (E. coli), thereby decreasing the risk of infection. Lactoferrin has antiviral activity [against human immunodeficiency virus (HIV), herpes simplex virus (HSV), cytomegalovirus (CMV)]. It is also anti-adhesive for E coli and anti-invasive for Shigella. In addition it also has Immuno-modulating and anti tumor activity⁶ and favours the intestinal cell growth.7-9

- Secretory IgA (sIgA): This is the most abundant immunoglobulin present in breast milk, which is resistant to digestion and aids in boosting an infant's immature immune system.
- Lysozyme in breast milk: It helps in the degradation of the outer cell wall of gram-positive bacteria and inactivates gram-negative bacteria in the presence of lactoferrin. It is also known to exhibit antiamoebic and anti-HIV activity.
- Haptocorrin: Haptocorrin helps in absorption of vitamin B₁₂ in early life by binding with it. It resists digestion and has antimicrobial activity.

- Amylase: It aids in the digestion of oligosaccharides and polysaccharides in breast milk. It may also have an antibacterial function by breaking down the polysaccharides of the bacterial cell wall.^{6,7}
- *Casein:* It is a phosphoprotein and is present in milk as a complex molecule, along with calcium and phosphate called casein micelles. Casein in human milk is easily digestible and helps in the growth and development of the child.^{10,11}
- *Human milk mucins:* They are glycoproteins and the majority of them are present in the milk fat globular membrane. They inhibit pathogens binding to the host cell surface (specifically rotaviruses and binding of S-fimbriated *E. coli* to buccal epithelial), also involved in the inhibition of viral replication, thus offering protection against infections.^{12,13}
- Carbohydrate components of breast milk: They include lactose and HMOs. The lactose content of breast milk is 7 g/dL, and is the major source of energy. Lactose provides galactose which acts as a substrate for gangliosides and cerebrosides synthesis in the brain. HMOs are the natural prebiotics in the breast milk and play an important role in the protection of infants against infections. HMOs prevent adhesion and invasion of microorganisms across the intestinal wall. In addition, HMOs also provide sialic acid which is an important constituent of gangliosides and plays an important role in neurodevelopment.¹⁴⁻¹⁷
- Fat components of human milk: They are mainly triglycerides, free fatty acids, and cholesterol. *Triacylglycerol* is the major

component of fat in breast milk (98–99%) and is another major source of energy. Human milk is a rich source of essential *fatty acids* such as linoleic acid, linolenic acid, and their long-chain derivatives [long-chain polyunsaturated fatty acids (LCPUFAs)], namely docosahexaenoic acid (DHA) and arachidonic acid (AA), which play an important role in the development of retinal and neural tissue. The preformed *cholesterol* in breast milk helps in delivering the fatty acids to membranes and target organs of the infant.¹⁸

Micronutrients

Breast milk contains vitamins (both water and fat-soluble) and minerals. Breast milk is rich in water-soluble vitamins, namely thiamin, riboflavin, vitamins B_6 and B_{12} , and fat-soluble vitamin A. Minerals and a multitude of trace elements are present in breast milk (copper, zinc, barium, cadmium, chromium, cobalt, cerium, lanthanum, manganese, molybdenum, nickel, lead, rubidium, tin, and strontium), which have high bioavailability. Vitamin D and K are found in very low concentrations in human milk.^{6,7}

Vitamin A in human milk is present mainly as retinyl palmitate, whose primary function is in cell differentiation, epithelium formation, retinal tissue development, and antioxidant action.^{6,7} Carotenoids in human milk are mainly lutein, lycopene, and zeaxanthin, which help in neural and retinal tissue synthesis and play an important role in an infant's cognition and early memory.¹⁹

Vitamin D is essential for calcium absorption, bone and teeth mineralization, neurodevelopment, and immune function,⁶ which needs to be supplemented (400 IU/day from birth) as its concentration in human milk is very low.²⁰

Colostrum contains a high concentration of vitamin E, an antioxidant that plays an important role in immune function. The concentration of vitamin E decreases as the maturation of milk advances and gets stabilized after 4 weeks of the postpartum period.⁶

Human milk is deficient in vitamin K and therefore, newborns are at higher risk for hemorrhagic diseases. Hence, every newborn child should receive a prophylactic dose of vitamin K at birth.⁶

Vitamin B_{12} is essential for folate metabolism and deoxyribonucleic acid (DNA) synthesis. In human milk, vitamin B_{12} is tightly bound to apo-haptocorrin, a cobalamin-binding protein. Folic acid is necessary for protein and DNA and ribonucleic acid (RNA) biosynthesis and its requirement is greatest in the phase of growth and development.⁶

Choline is an important nutrient present in human milk; it plays a crucial role in fetal and infant development. Total choline concentrations in breast milk increase rapidly between the 7- and 22-day postpartum period and remain relatively stable in mature milk.²⁰ This serves as a precursor for the synthesis of acetylcholine, phospholipids (critical for cell membrane), surfactants (important in lung maturity), and bile formation. Choline also has a significant role in brain development, along with DHA and lutein; further, it can influence infant memory.²¹⁻²³

Vitamin C in human milk functions as an antioxidant and immune modulator. It stimulates leukocytes, augments antibody production, and enhances the synthesis of interferons.⁶

The iron content in human milk is relatively low but has high bioavailability. Iron plays an important role in the synthesis of hemoglobin, the formation of new tissue, immune function, and cognitive and neurodevelopment.¹⁸ In preterm babies, since the iron reservoir is not sufficient, it has been recommended to supplement iron in the dose of 2–4 mg/kg/day starting at 2 weeks and continuing until 6 months of age.²⁴

In human milk, copper is bound with ceruloplasmin and it plays an important role in cellular respiration, iron metabolism, and connective tissue synthesis.⁶ Breast milk is relatively deficient in zinc, but it is absorbed efficiently (high bioavailability). It takes part in cell differentiation, especially in tissues that are characterized by rapid turnover and proliferation such as the immune cellular network and the gastrointestinal system.¹⁸ Zinc deficiency in infants results in stunted growth and compromised immune functions, resulting in high morbidity and mortality associated with diarrhea and respiratory infections.⁶

The iodine in breast milk is essential for thyroid hormone synthesis, neurodevelopment, infant growth, and survival. Similarly, selenium in human milk is a component of a potent antioxidant glutathione peroxidase.^{6,18}

Minerals

Predominant minerals present in human milk include calcium, phosphorus, sodium, potassium, chloride, and magnesium.

Both calcium and phosphorus are essential for bone and teeth mineralization. Magnesium plays a structural role in bone and is involved in more than 300 essential metabolic reactions.⁶ Other major minerals such as sodium, potassium, and chlorides are adequate in human breast milk **(Table 1)**.

Bioactive Factors²⁷

Varieties of *cytokines* [tumor necrosis factor (TNF), interleukin 1 (IL-1), IL-6, and

Proteins Carbohydrates Fats/lipids Micronutrients /itamin A /itamin D /itamin E /itamin K /itamin B ₁	Per deciliter 0.9 g 7 g 3.5 g 61 μg 0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg 0.18 mg
Carbohydrates Fats/lipids Wicronutrients Vitamin A Vitamin D Vitamin E Vitamin K Vitamin B ₁	7 g 3.5 g 61 μg 0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg
Fats/lipids A Micronutrients /itamin A /itamin D /itamin E /itamin K /itamin B ₁	3.5 g 61 μg 0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg
Micronutrients /itamin A /itamin D /itamin E /itamin K /itamin B ₁	61 μg 0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg
/itamin A /itamin D /itamin E /itamin K /itamin B ₁	0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg
/itamin D /itamin E /itamin K /itamin B ₁	0.1 g 0.08 mg 0.3 μg 0.01 mg 0.04 mg
/itamin E /itamin K /itamin B ₁	0.08 mg 0.3 μg 0.01 mg 0.04 mg
/itamin K /itamin B ₁	0.3 μg 0.01 mg 0.04 mg
/itamin B ₁	0.01 mg 0.04 mg
	0.04 mg
/itamin B ₂	5
-	0 18 mg
/itamin B ₃	errering
/itamin B ₅	0.22 mg
/itamin B ₆	0 mg
/itamin B ₁₂	0.05 μg
olate	5 µg
Choline	15.76 mg
ron	0.03 mg
Zinc	0.16 mg
Copper	0.04 mg
Selenium	1.76 µg
Manganese	0.04 mg
Minerals	
Calcium	31.6 mg
Phosphorus	13.6 mg
Magnesium	3 mg
Sodium	16.8 mg
Potassium	50 mg
Chloride	43 mg

IL-8, interferon-gamma (IFN), and transforming growth factor beta (TGF- β)], *adipokines* (leptin, adiponectin, resistin, ghrelin, obestatin, nesfatin, and apelin), immunoglobulins (sIgA), *antioxidants* (betacarotenes, tocopherols, lycopene, glutathione, melatonin, superoxide dismutase, catalases, and glutathione peroxidases), and growth factors (GF) (epidermal GF, neuronal GF, insulin-like GF (IGF), vascular endothelial GF, etc.) are found in human breast milk (Tables 2 and 3).

Human Milk Cells

Breast milk contains white cells, stem cells, and mesenchymal cells, which are involved in the development of immune cells that help in tissue repair.³³ Stem cells have the potential to reprogram into multiple tissue types.³⁴

Micro-RNAs

Human milk is particularly rich in micro-RNAs, which are potentially involved in the development and maturation of a child's immature immune system.³⁵ Micro-RNAs are small noncoding RNA molecules that regulate gene expression at the posttranscriptional level, modulating several cell functions such as cell cycle, proliferation, differentiation, apoptosis, immune response, and neurodevelopment.^{11,36}

Exosomes: They are extracellular microvesicles derived from mammary glands that contain micro-RNAs with the size of around 22 nucleotides. These exosomes combine with micro-RNAs and prevent their digestion in infants' gut, thus allowing the micro-RNAs to exert their function. Human milk exosomes can be protective against the development of intestinal injuries, such as necrotizing enterocolitis (NEC).³⁷

HAMLET

Human alpha-lactalbumin made lethal to tumor cells (HAMLET) in human milk is a complex made up of alpha-lactalbumin and oleic acid that induces cell death in tumor cells but not in normal cells. HAMLET kills a wide range of malignant cells, which

TABLE 2: Major constituents in human colostrum and transitional and mature milk ²⁸ (average per 100 mL).							
	Colostrum	Transitional milk	Mature milk				
Energy (kcal)	58	74	71				
Lactose (g)	5.3	6.6	7.0				
Casein (g)	1.2	0.7	0.4				
Fat (g)	2.9	3.6	3.8				
Minerals							
Calcium	31	34	33				
Magnesium	4	4	4				
Potassium	74	64	55				
Sodium	48	29	15				
Iron	0.09	0.04	0.15				

TABLE 3: Bioactive compone	
Bioactive components	Functions
Antimicrobial: • Lactoferrin • Lactadherin • Lysozymes • Casein	 Acute phase protein, chelates iron, antibacterial, antioxidant Antiviral enhances phagocytosis of apoptotic cells Bacterial lysis, immunomodulating activity, and binding to bact lipopolysaccharide—reduce the endotoxic effect^{6,7} Antiadhesive for H pylori, H influenza, and S pneumonia The strong growth-promoting factor for B bifidium¹¹
Immunoglobulins: • slgA • lgG • lgM	 Pathogen-binding inhibition Phagocytosis, anti-inflammatory, antimicrobial, etc. Agglutination, complement activation
Immunomodulators: • Cytokines - IL-1 - IL-6 - IL-7 - IL-8 - IL-10 - INF-γ - TNF-β - TNF-β - TNF-α • Chemokines • Cytokine inhibitors	 Production of defense agents in mammary glands Acute phase response, B-cell activation Increased thymic size and output Recruitment of neutrophils and proinflammatory Repressing inflammation, antibody production Proinflammatory Anti-inflammatory, stimulation of T cell Inflammatory immune activation Prevents macrophage movement Inhibition of TNF-alpha, anti-inflammatory
Growth factors: • EGF • HB-EGF • VEGF • NGF • IGF • Erythropoietin	 Stimulates cell proliferation and maturation Protection against hypoxia and ischemia Promotion of angiogenesis and tissue repair Promotion of neuron growth and maturation Stimulation of growth and development, RBCs, and Hb Erythropoiesis, intestinal development

Contd...

Bioactive components	Functions
Hormones: • Calcitonin • Somatostatin • Cortisol • Insulin • Thyroid hormone	 Development of enteric neurons Regulation of gastric epithelial growth Physiological stress response, immunity, metabolism³⁰ Gut maturation in the infant³¹ Regulate breathing, heart rate, BMR, growth, and development³²
Metabolic hormones: • Adiponectin • Leptin • Ghrelin	 Reduction of baby's BMI and weight, anti-inflammatory Regulation of energy conversion and appetite Regulation of energy conversion and appetite
Oligosaccharides: • HMOs • Gangliosides • Glycosaminoglycans	 Prebiotics, stimulate beneficial colonization Anti-inflammatory Brain development, anti-infective/antiadhesive
Mucins	Blocks infections by viruses and bacteria
Cells: • Macrophages • Stem cells • Probiotic bacteria • HAMLET cells	 Protection against infection, T-Cell activation Regeneration and repair Probiotic Anticancer cells
Genetic materials: • Micro-RNAs	 Infant protection and development Modulates cell cycle, proliferation, differentiation, apoptosis, and immune response and neurodevelopment^{11,36}
Exosomes	 Protects against the development of intestinal injury such as that seen in NEC³⁷

(BMI: body mass index; BMR: basal metabolic rate; EGF: epidermal growth factor; HAMLET: human alphalactalbumin made lethal to tumor cells; Hb: hemoglobin; HB-EGF: heparin-binding EGF; HMOs: human milk oligosaccharides; IgG: immunoglobulin G; IGF: insulin-like growth factor; IL: interleukin; INF-γ: interferon gamma; NEC: necrotizing enterocolitis; NGF: neuronal growth factor; RBCs: red blood cells; RNAs: ribonucleic acid; sIgA: secretory IgA; TNF: tumor necrosis factor; VEGF: vascular endothelial growth factor)

explains why no tumors are seen in breast milk-fed babies.³⁸

Human Milk Microbiota

Breast milk is a natural source of prebiotic (HMOs) and probiotic (microbiota) such as *Bifidobacterium breve (B. breve), B. adoles-centis, B. longum, B. bifidum,* and *B. dentium*) which play a major role in the gut priming, thus offering protection.^{39,40}

UNIQUE FEATURES OF HUMAN MILK⁴¹

Human milk contains alpha-lactalbumin, whereas cow's milk contains more β -lactoglobulin.

The casein component of human milk is easily digestible as compared to cow's milk. The ratio between casein to whey protein in human milk is 40:60, whereas it is 80:20 in cow's milk. Human milk has high polyunsaturated fatty acid (PUFA) content compared to cow's milk. The fatty acids namely AA and DHA are high in human milk, both of which are essential for brain development and functioning, whereas cow's milk does not contain these fatty acids.

Human milk oligosaccharides (10-20 g/L) are specific to the baby, whereas cow milk oligosaccharides (1 g/L) are nonspecific.

Although iron and zinc levels are relatively low in human milk, they have

good bioavailability. Human milk has a relatively low concentration of vitamins D and K.

Infants of other mammals need to grow quickly; hence, more protein is in their milk than in human milk. Human infants need to develop their brains and nerves quickly, therefore providing less protein and more fat in human milk.⁴² The detailed nutritional composition of other animals' milk is compared with human milk in **Table 4**.

TABLE 4: Nutritional composition of different types of milk of animal origin.									
			Comp	osition in	100 g				
	Human	Cow	Buffalo	Goat	Sheep	Yak	Horse	Donkey	Camel
Energy (kcal)	70	62	99	66	100	100	48	37	76
Water (g)	87.5	87.7	83.2	87.7	82.1	82.6	89.8	90.8	84.8
Total protein (g)	1.0	3.3	4	3.4	5.6	5.2	2	1.6	3.9
Total fat (g)	4.4	3.3	7.5	3.9	6.4	6.8	1.6	0.7	5
Lactose (g)	6.9	4.7	4.4	4.4	5.1	4.8	6.6	6.4	4.2
Minerals:									
Calcium (mg)	32	112	191	118	190	129	95	91	154
lron (mg)	0.1	0.2	0.3	0.1	0.6	0.1			
Magnesium (mg)	3	11	12	14	18	10	7	4	8
Phosphorus (mg)	14	91	185	100	144	106	58	61	132
Potassium (mg)	51	145	112	202	148	95	51	50	186
Sodium (mg)	17	42	47	44	39	29	16	22	66
Zinc (mg)	0.2	0.4	0.5	0.3	0.6	0.9	0.2	0	0.7
Copper (mg)	0.1				0.1	0.1	0.1	0	
Selenium (g)	1.8	1.8		1.1	1.7	11			
Manganese (g)		8		18	18				
Vitamins:									
Retinol (g)	60	35	69	45	64				
Carotene (g)	7	16		13					
Vitamin A (gRE)	61	37	69	48	64				97
Vitamin E (mg)	0.08	0.08	0.19	0.05	0.11				0.15
Thiamine (mg)	0.01	0.04	0.05	0.06	0.07		0.03	0.06	0.01

Contd...

Composition in 100 g									
	Human	Cow	Buffalo	Goat	Sheep	Yak	Horse	Donkey	Camel
Riboflavin (mg)	0.04	0.2	0.11	0.13	0.34		0.02	0.03	0.12
Niacin (mg)	0.18	0.13	0.17	0.24	0.41		0.07	0.09	
Vitamin B ₅ (mg)	0.22	0.43	0.15	0.3	0.43				
Vitamin B ₆ (mg)		0.04	0.33	0.05	0.07				0.05
Folate (g)	5	8.5	0.6	16					
Biotin (g)		2	13	2.5	2.5				
Vitamin B ₁₂ (g)	0.05	0.51	0.4	0.07	0.66				
Vitamin C (mg)	5	1	2.5	1.1	4.6		4.3		3
Vitamin D (g)	0.1	0.2		0.1	0.2				1.6

Contd...

Source: Food and Agriculture Organization (FAO) of the United Nations.²⁵

KEY MESSAGES

- Breast milk contains macronutrients, micronutrients, minerals, and bioactive substances.
- Breast milk is a dynamic living fluid, species-specific, baby-specific, and tailor-made.
- The colostrum, in addition to nutritional superiority, also has bioactive substances which play an important role in the prevention of infections in infants and hence is called natural vaccine.
- Breast milk has genetic materials which play an important role in inheritance and immune and tissue maturation.
- Breast milk has two components foremilk which is white, watery, and rich in proteins that satisfies thirst and hindmilk which is yellowish, thick, and rich in fats satisfies hunger.
- Breast milk secretion is influenced by the gestational age, time of the day, frequency and efficacy of extraction, and psychological status of the mother.

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Art and Science of Breastfeeding & Beyond

"Breastfeeding is the essence of life to the mother and baby." It lays a good foundation for a long quality of life. It is also the right of the mother and baby. Hence, it is essential to ensure a conducive environment for the mother and baby for the establishment of successful lactation.

Though most women wish to breastfeed their infants, they often face difficulties at the initiation and maintenance of breastfeeding. Therefore, they need to be provided with skilled practical assistance and support. There is a huge knowledge gap among the healthcare professionals regarding optimal infant and young child feeding practice.

This book provides comprehensive state-of-the-art information on optimal infant and young child feeding including the basics of human lactation, problems of breastfeeding and solutions, breastfeeding in special circumstances, research in lactation and ways to enhance breastfeeding rates across the nations.

This book is intended to empower healthcare professionals, medical and nursing students, and breastfeeding support groups to improve their knowledge and wisdom which in-turn is hoped to aid in enabling breastfeeding women.

This book is reader-friendly and hopes to help healthcare professionals, healthcare workers, medical and nursing students, practitioners, mother support groups, and the public at large.

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Printed in India

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