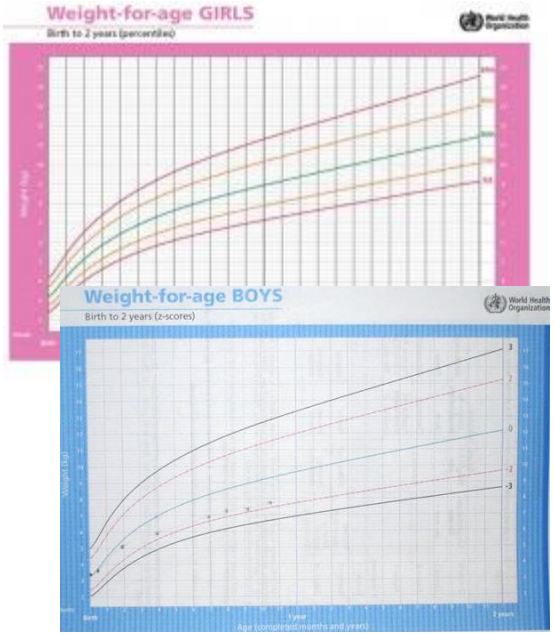
Wyeth Nutrition
SCIENCE CENTER

Bioactive nutrition

The role of biological and functional components of milk on infant development

Ryan Carvalho

Breastfeeding is the ideal way to feed an infant



Respiratory

- Upper respiratory tract infection
- Lower respiratory tract infection
- Asthma
- RSV bronchiolitis

Gastrointestinal

- Necrotizing enterocolitis (NEC)
- Gastroenteritis
- Celiac disease
- Inflammatory bowel disease

Other

- Otitis media
- Atopic dermatitis
- Type 1 and 2 diabetes
- Obesity
- Childhood leukemia
- SIDS

Pediatrics 2012;129:e827–e841

Learning from mother's milk for infant nutrition and health

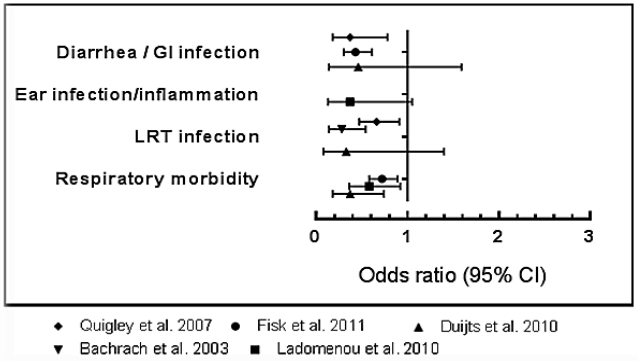
Breastfeeding is associated with lower risk of:[1–3]

Respiratory and gastrointestinal infections

Obesity and diabetes

Possibly allergies, but needs clarification

Exclusive breastfeeding (6m) *versus* no breastfeeding [4]



➡ ***Suggests involvement of breast milk-specific components***

1. Victora CG, et al. Lancet 2016;387:475–90; 2. Victora CG, et al. Am J Clin Nutr 2016;70:1420–7; 3. Lodge CJ, et al. Acta Paediatr 2015;104:38–53. 4. Forest plot drawn from indicated publications

Components of Human milk : Nutritive and Functional

- 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.
- Varied bioactive factors, which include cells, anti-infectious and anti-inflammatory agents,growth factors, and prebiotics.
- COLOSTRUM
  - 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
  - lactose, indicating its primary functions to be immunologic and trophic rather than nutritional

Pediatr Clin North Am. 2013 February ; 60(1): 49–74. Human Milk Composition: Nutrients and Bioactive Factors

Functional benefits of bioactive components in milk

These components provide enzymatic activity; inhibit proteolytic enzymes; stimulate neonatal intestinal, immune, and brain development; shape the microbiome; and protect the infant from infection.

The diversity of human milk proteins differentiated and significantly wider.

Some of these differentially expressed proteins with greater abundance in human milk, including lactoferrin, polymeric immunoglobulin receptor, alpha-1antichymotrypsin, vitamin D-binding protein, and haptocorrin.

Importantly, the functions of proteins that were more abundant in human milk were associated with development of the gastrointestinal tract, the immune system, and the brain.

Nutriton components of human milk

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	2.2 (1.3, 3.3)	4.4 (2.6, 6.2)	7.6 (6.4, 8.8)	78 (61, 94)
Born 32-33 weeks, n=20	1.9 (1.3, 2.5)	4.8 (2.8, 6.8)	7.5 (6.5, 8.5)	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)

- US, Danish, Austrailian cohort
- Variability
  - Maternal factors
  - Duration post partum
  - Gestational age
  - Collection factors

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

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More recent breast milk composition

Author (year), n, location	Protein (g/100 mL) mean ± S.D.	Lipids (g/100 mL) mean ± S.D.	Lactose (g/100 mL) mean ± S.D.	Energy (kcal/100 mL) mean ± S.D.
Term infants, mature milk, single breast full collection				
Thakkar et al., (2013), + Lee et al., (2016) 50, Singapore	1.38 0.30	4.65 ± 2.10	6.44 ± 0.69	70.24 ± 22.00
Yang et al., (2014), 540, China	1.10 ± 0.20	3.50 ± 1.60	7.20 ± 0.30	62.00 ± 14.00
Fischer Fumeaux et al., (2010) 25, Switzerland	1.70 ± 0.30	1.65± 0.60	5.80 ± 0.10	53.10 ± 8.80
Preterm infants, mature milk, single full breast collection				
Fischer Fumeaux et al., (2010) 25, Switzerland	1.50 ± 0.50	1.63± 0.53	5.90 ± 0.20	58.70 ± 10.20

Nutritive components of human milk

- The most abundant proteins are casein, α-lactalbumin, lactoferrin, secretory immunoglobulin IgA, lysozyme, and serum albumin.
- 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.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 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
- Micronutrients vary in human milk depending on maternal diet and body stores including vitamins A, B1, B2, B6, B12, D, and iodine. Regardless of maternal diet, Vitamin K is extremely low and Vitamin D also occurs in low
- quantities in human milk

Bioactive components of human milk

- 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”
  - some are produced and secreted by the mammary epithelium,
  - some are produced by cells carried within the milk,
  - others are drawn from maternal serum and carried across the mammary epithelium by receptor-mediated transport.

Schrezenmeir J, Korhonen H, Williams C, Gill HS, Shah N. Foreword. The British journal of nutrition. 2000;

Components of Human milk : Functional

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; C

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

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Components of Human milk : Functional

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; Corthésy, 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; Erbağci, 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

Factors that influence bioactive components in breast milk

Maternal factors

- Maternal genotype
- Maternal diet
- Maternal physiologic state
- Mode of delivery
- Infant gestational age
- Lactation stage
- Underlying medical conditions
- Maternal medication use
- BMI maternal

Infant clinical parameters

- **Infectious morbidity**
- **Allergies**
- Growth and body composition
- Cognitive development
- Gastrointestinal developments
- Immune system maturation
- metabolism

Bindl, Austria, 2007, unpublished results

Bioactive functions of human milk components.

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

EGF is critical to the maturation and healing of the intestinal mucosa.

- 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. BDNF can enhance peristalsis.

- Tissue growth: IGF-1 may also play a role in the survival of enterocytes following intestinal damage from oxidative stress.

Bioactive functions of human milk

- Growth-regulating hormones: Calcitonin and somatostatin
- Regulating metabolism and body composition: Adiponectin and other hormones
  - Adiponectin is a large, multi-functional hormone that actively regulates metabolism and suppresses inflammation.
  - Some have proposed that adiponectin in human milk may contribute to reduced incidence of overweight and obesity in later life.
  - Adiponectin is a large, multi-functional hormone that actively regulates metabolism

Immunologic functions of milk bioatives

- Cells of human milk: Human milk contains a variety of cells, including macrophages, T cells, stem cells, and lymphocytes.
- Levels of cell differ in children with certain allergy
- 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.
- Stem cells have also been identified in human milk; their function is under investigation

Immunologic factors in human milk

- Milk-borne TGF-β regulates inflammation and wound repair, and helps prevent allergic diseases.
- Granulocyte-colony stimulating factor (G-CSF), identified in human milk decades ago, has beneficial effects on intestinal development and the treatment of sepsis. It acts at the intestinal surface, where it increases villi, crypt depth, and cell
- The colostrum of allergic mothers contains lower IFNγ but higher Th2 cytokines IL-4 and IL-13 compared to non-allergic mothers.

Protection from Infections

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.

While sIgA is the predominant antibody of human milk, milk also contains IgM and IgG, the latter becoming more abundant in later.

Lactoferrin, an iron-binding glycoprotein belonging to the transferrin family, which is effective against many different bacteria, viruses, and fungi.

Lactadherin, prevents rotaviral infection in the newborn. Following infection or damage, lactadherin mediates phagocytic uptake of apoptotic cells and stimulates a signaling cascade that results in decreased inflammation and promotes healing during intestinal

Another multi-functional protein, bile salt stimulating lipase (BSSL) also protects infants from viral infection, including Norwalk.

Secretory IgA (sIgA)

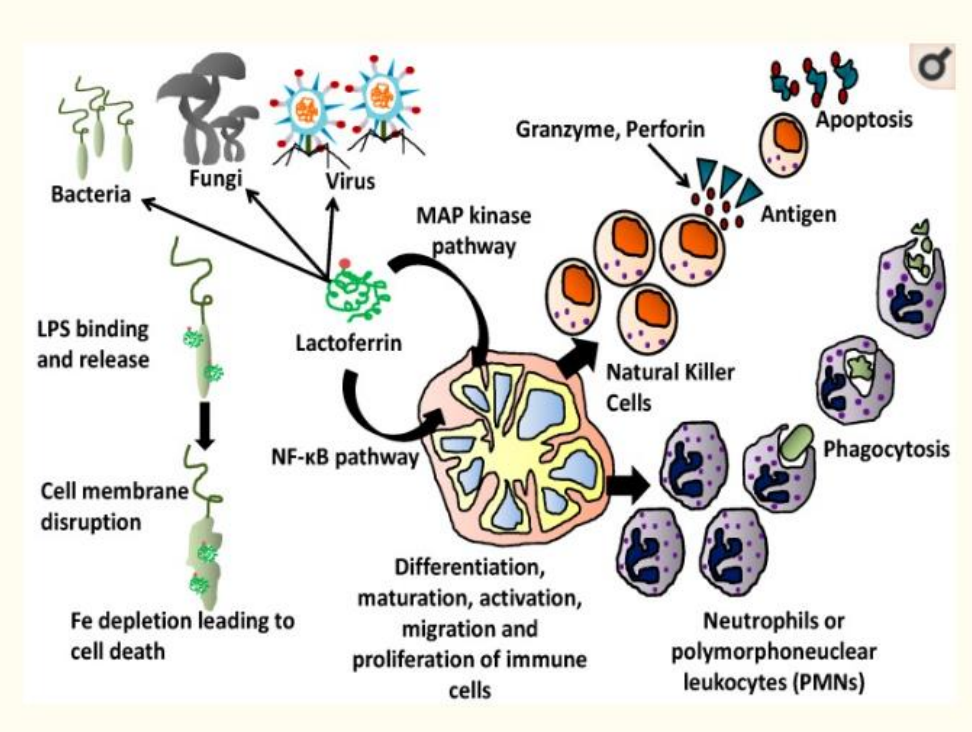
SIgA is an antibody that plays a critical role in immune function in the mucous membranes. More IgA is produced in mucosal linings than all other types of antibody combined; between three and five grams are secreted into the intestinal lumen each day.

Role of sIgA

Secretory IgA (SIgA) serves as one of the early line of defense in protecting the intestinal epithelium (the gut lining) from enteric toxins and pathogenic (disease causing) microorganisms. It does this by interfering with the earliest steps in the infection process by virtue of its ability to block toxins and pathogens from adhering to the intestinal epithelium (gut lining). Ability of Secretory IgA to promote the clearance of antigens and pathogenic microorganisms from the intestinal lumen by blocking their access to epithelial receptors, entrapping them in mucus, and facilitating their removal.

Please add gif or secretory IgA

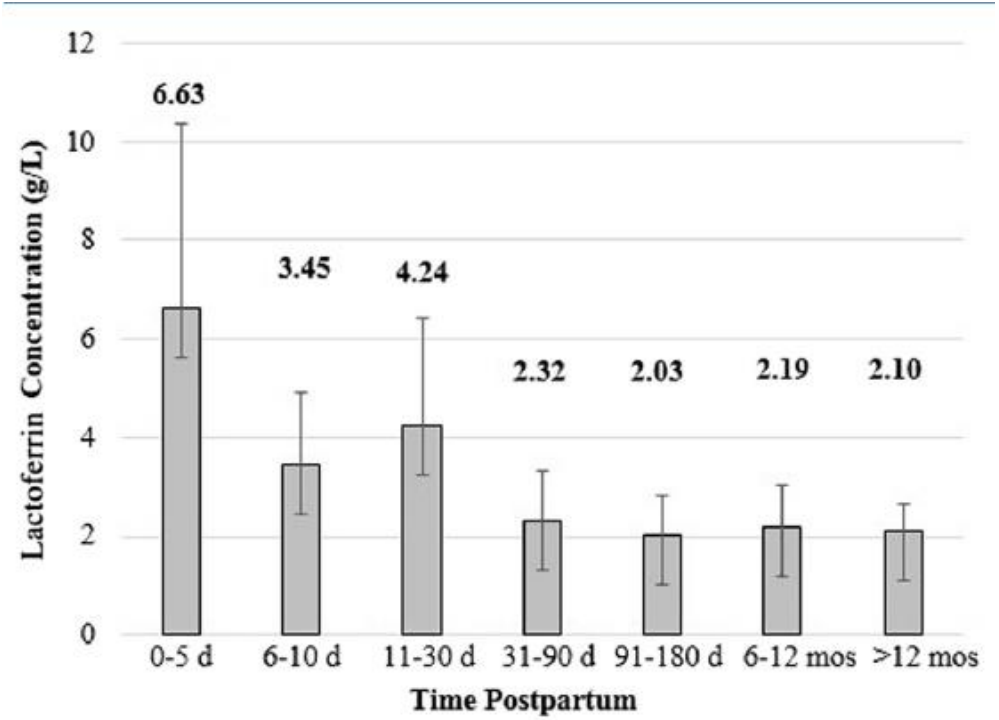
Lactoferrin



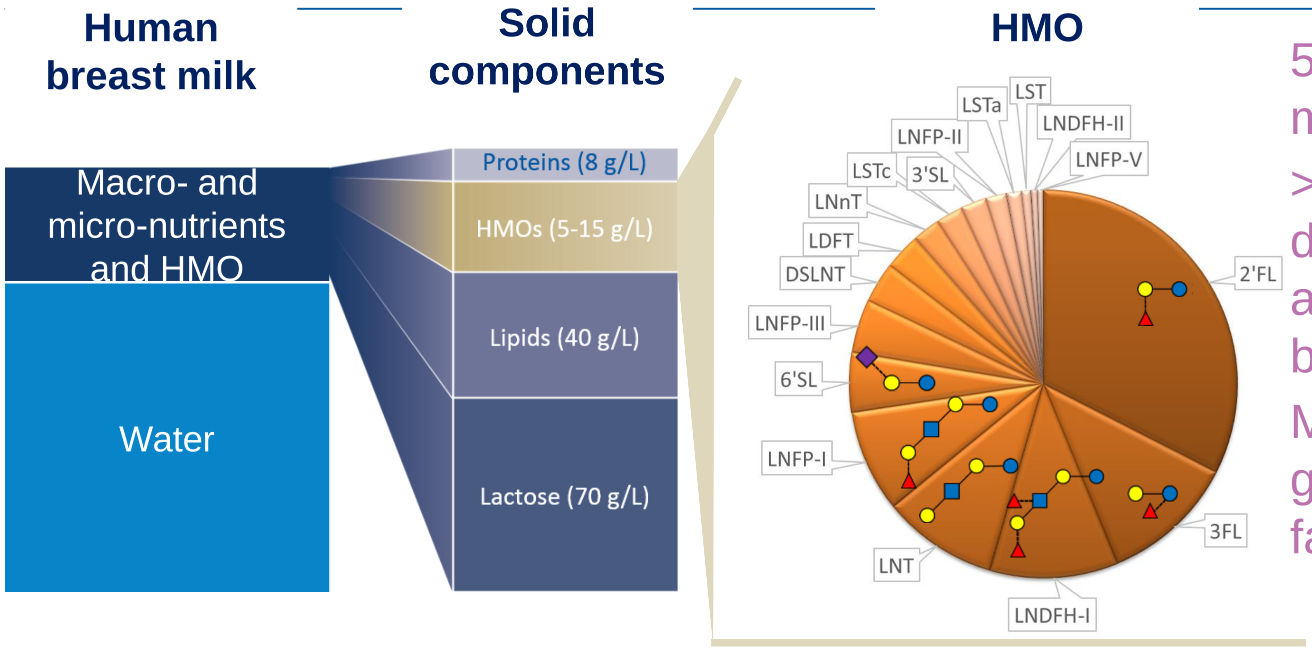
Role of lactoferrin in the activation of immune cells. Lactoferrin enters in the intestinal microvilli through the help of lactoferrin receptors and transferrin receptors present on the mucosal surface of the intestinal cells. The lactoferrin molecule further boosts up the immune response due to IFN-γ, TNF-α, IL-6 and by activating NK cells, PMNs and CD3+ and CD4+ T cells. Finally the lactoferrin enters the cells by receptor mediated endocytosis where it is released within the cells once the receptors are digested by endosomes. The lactoferrin induces release of cytochrome C from mitochondria which further activates caspase 3 to cause apoptosis in tumour cells

Molecules. 2015 Jun; 20(6): 9703–9731.

Lactoferrin concentration in human milk



Gross composition of breast milk



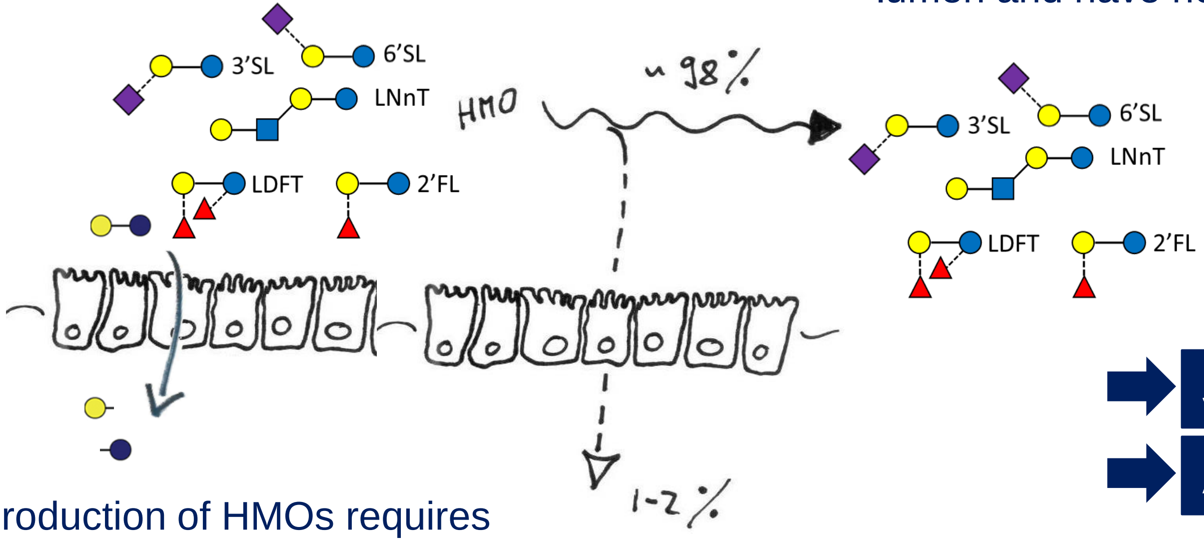
- 5 to 15 g/L in breast milk
- >130 structures described, of which about 15 make up the bulk (>80%)
- Most HMO are not generally present in farmed animal milks

(Drawn from compiled data of Austin, 2016; Sprenger, 2017; Kunz, 2017 and Austin et al. NRC unpublished data)

Zivkovic AM, et al. ... 108(Suppl. 1):4653–8; Austin S, et al. Nutrients 2016;8:pii: E346; Sprenger N, et al. PLoS One 2017;12:e0171814; Kunz C, et al. J Pediatr Gastroenterol Nutr 2017;...
Bode L. Glycobiology 2012;22:1147–1162.

HMO biology

The majority of HMOs remain intact in gut lumen and have no nutritive value



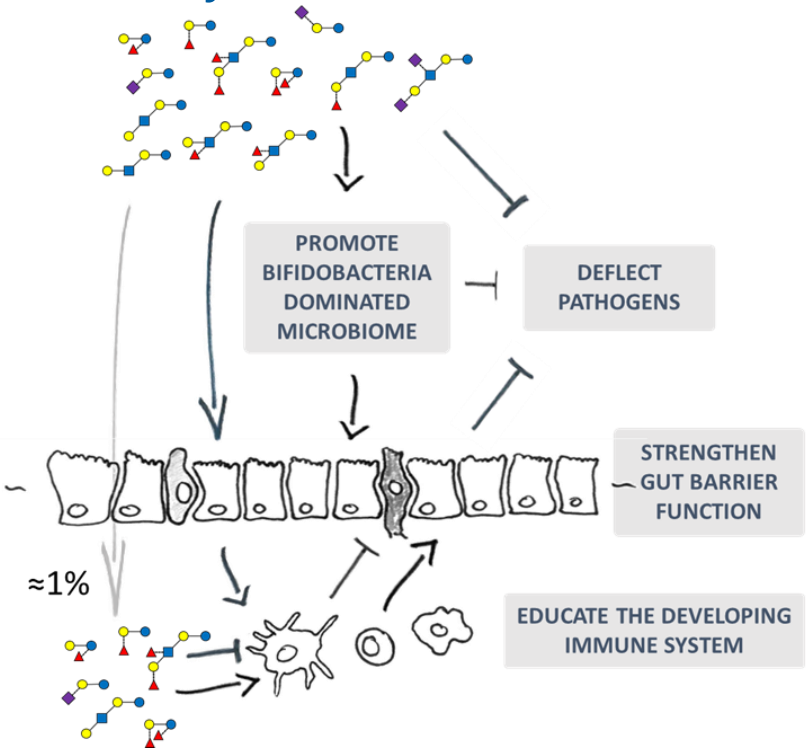
Production of HMOs requires "mother's tremendous expenditure of energy", yet,

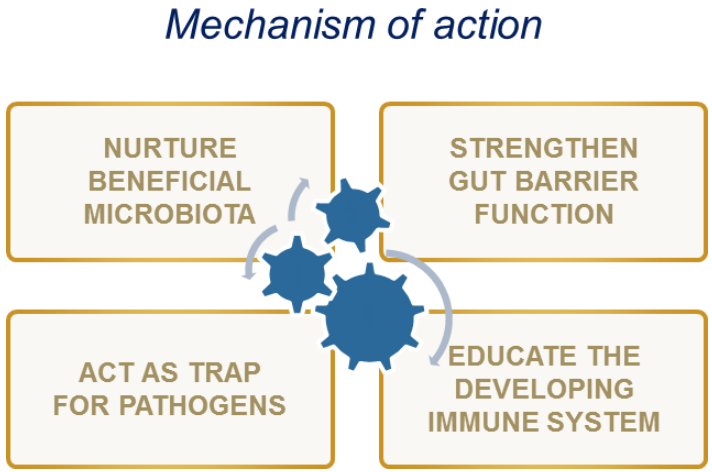
So what do HMOs do?

And, what do BMOs do?

Ruhaak L, et al. Anal Bioana Chem 2014;406:5775–84. Underwood MA, et al. Pediatr Res 2015;77:229–35.

Summary: HMO, a multifunctional breast milk component





Osteopontin in bovine milk

The concentration in bovine milk is approximately 18 mg/l

In human breast milk the osteopontin concentration is significantly higher

Modification and cleavage patterns are similar in human and bovine milk

What is the function of osteopontin in milk?

Inhibitor of calcium precipitation/crystallization?

Part of the innate immune system?

-activates the cellular immune response

-binds bacteria and induces phagocytosis of these

Osteopntin levels in human milk

	Age of mother (years)	Time post partum (days)	OPN conc. (mg/l)	Total protein conc. (mg/l)	OPN/total protein (w/w(%))
	28	58	66	8550	0.77
	35	13	257	8327	3.09
	28	12	201	13268	1.51
	25	6	65	11868	0.55
	31	9	46	15430	0.30
	25	12	22	11442	0.19
	30	33	102	10000	1.02
	31	39	114	10157	1.12
	30	17	67	10328	0.65
	23	12	212	10936	1.94
	30	10	105	12576	0.83
	31	27	157	5376	2.92
	30	17	185	4459	4.15
	31	37	103	5568	1.85
	30	10	239	12628	1.89
	22	15	100	6664	1.50
	27	26	100	9706	1.03
	30	14	102	3963	2.57
	35	24	322	9660	3.33
	32	15	18	2077	0.87
	32	22	47	1834	2.56
	35	28	171	5219	3.28
	28	28	192	4602	4.17
	30	17	245	8832	2.77
	27	22	154	3426	4.50
	30	11	206	4069	5.06
	29	15	175	4738	3.69
	37	15	40	6520	0.61
	27	28	203	11562	1.76
Mean	29.6	20.4	138	8062	2.1
SD	3.5	11.3	79	3680	1.4

High degreeof variation in osteopontin content: 18-322 mg/L
Average: 138 mg/L , ~ 2.1% of total protein

Osteopontin Summary of benefits

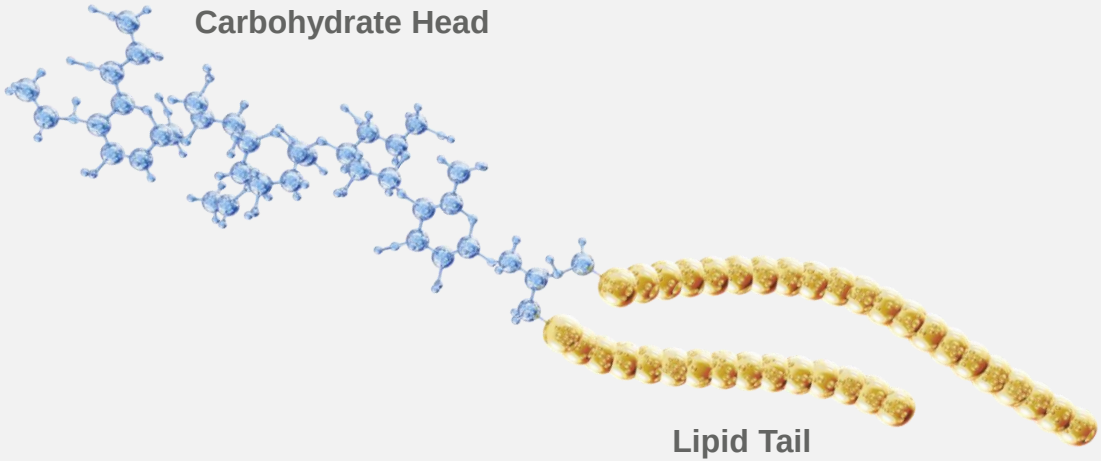
Osteopontin–

- ~138 mg/ml in humanmilk
- activatesthe cellularimmune system
- binds to bacteria
- binds to monocytes
- promotes monocyte migration
- inducesphagocytosisof bacteria

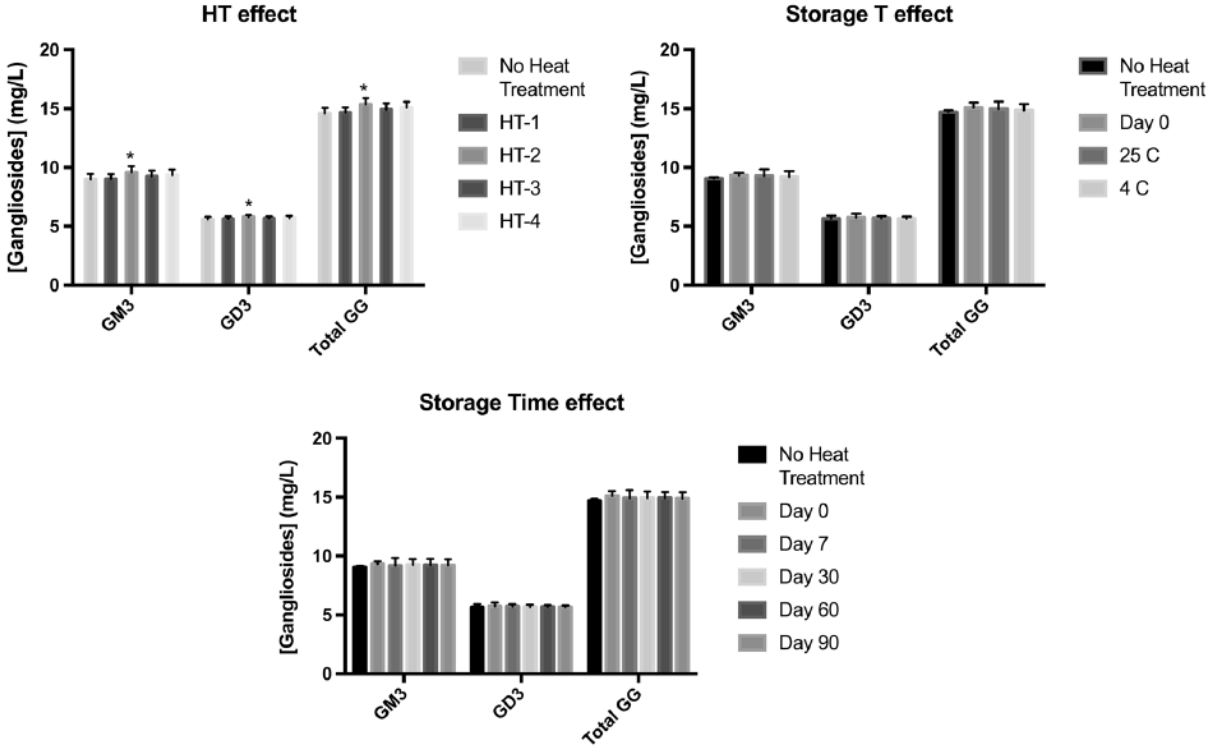
Gangliosides

Structure

- Complex molecule with a unique structure composed of[1]:
  - A complex carbohydrate: oligosaccharide linked to sialic acid
  - A lipid: sphingolipid (fatty acids plus sphingosine)



Effect of heat treatment and storage time on human milk gangliosides (GM3, GD3, Total, GG).



npj Science of Food (2018) 2:5

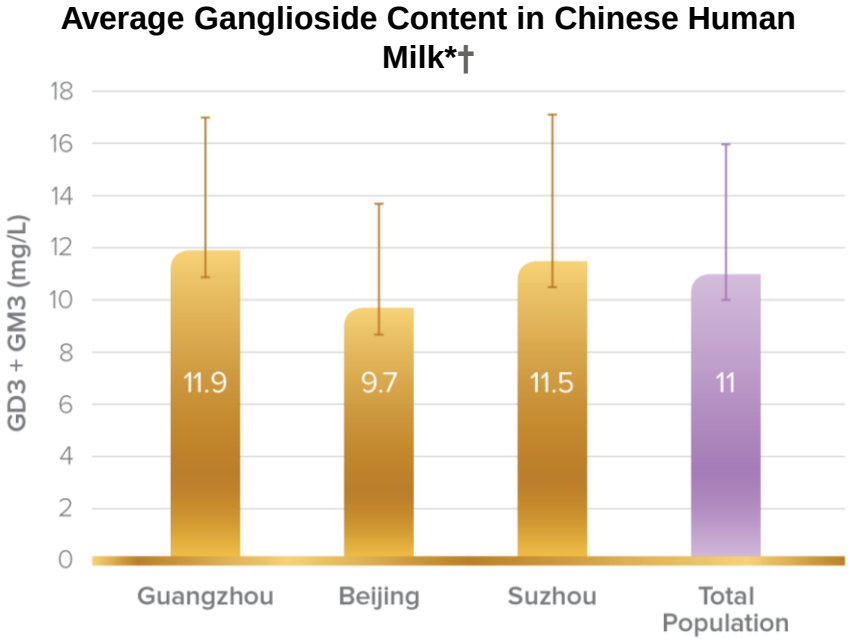
Breastmilk Naturally Contains Gangliosides

The two major gangliosides found in breastmilk are GD3 and GM3[1]:

GD3 is more predominant in colostrum

GM3 is more predominant in mature milk

The average ganglioside content is approximately 11 mg/L[2-4]



*Mature milk (N = 345).

†Levels are comparable to min/max values from other publications.[2-4]

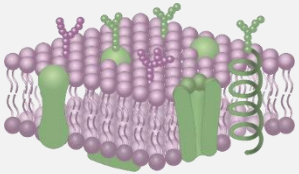
Gangliosides

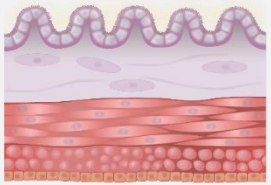
Structure

- Complex molecule with a unique structure composed of[1]:
  - A complex carbohydrate: oligosaccharide linked to sialic acid
  - A lipid: sphingolipid (fatty acids plus sphingosine)

Location in the Body

- Widely distributed throughout the body[2]:
  - Integral to the structure and function of cell membranes[2]
  - Abundant in the brain[3-6]
  - Found in the intestinal wall of the gut[3-8]





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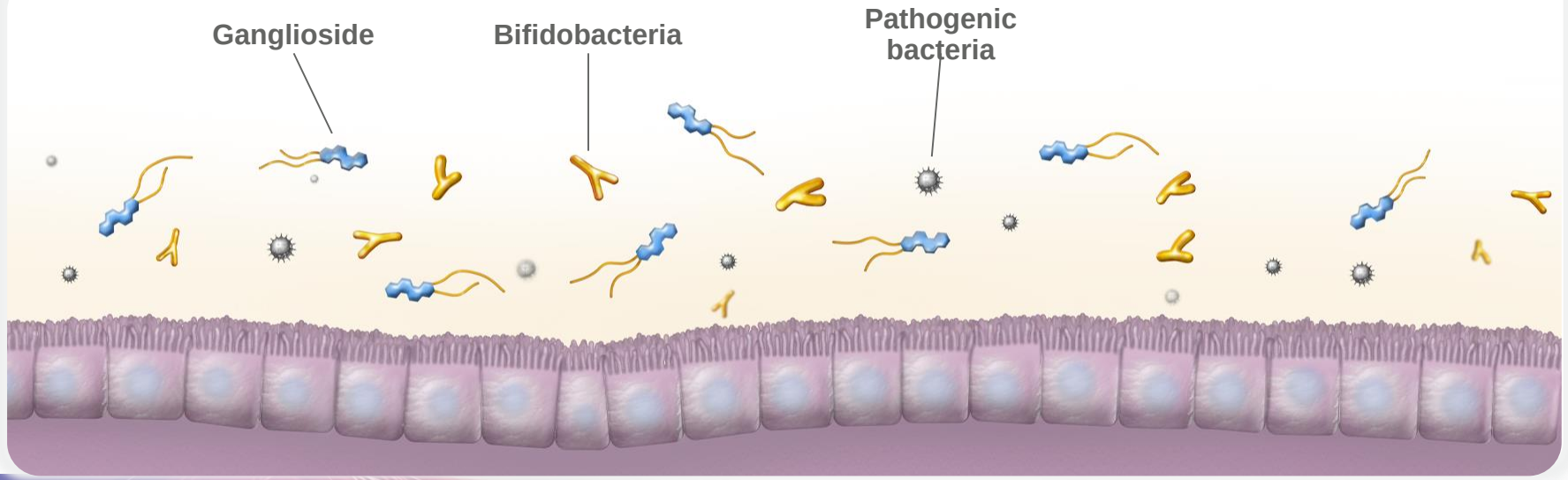
Source

- Produced endogenously (naturally by the body)[2,9]
- Obtained from the diet, such as through breastmilk[2,9]

Gangliosides Are Thought To Play An Important Role In Intestinal Immunity: Promote a Healthy Microbiota[1-7]

Resist digestion and reach the intestinal tract intact[8]

May guide the composition of the microbiota in the gut



Gangliosides Are Thought To Play An Important Role In Intestinal Immunity: Prevent Pathogen Adherence[1-7]

- The gut is a major site of contact with pathogens as it is a frontline barrier to limit pathogenic bacteria from invading the host[8-10]
- Pathogens want to attach, colonize, and invade the host—to attach, they bind to receptors in the gut[1-5]
- Gangliosides may act as decoys, as they look structurally similar to the receptors, interfering with pathogen binding[1-5]