Bioactive components of human milk

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Summary

Human milk is a unique, complex fluid containing a full range of nutrients and a wealth of bioactive, non-nutritional components such as antimicrobial and anti-inflammatory factors, cytokines, digestive enzymes, transport agents, hormones, trophic factors and growth modulators. This review briefly details these bioactive factors, discusses their putative mode of action and compares their concentration in human milk with that in cow's milk. Four examples illustrate the diversity of possible functions: secretory-IgA, amylase, bile-salt stimulated lipase (BSSL) and the feedback inhibitor of lactation (FIL). The physiological significance of individual bioactive components is difficult to evaluate, but there is compelling evidence that breast-feeding has many health benefits for the human infant.

Introduction

Human milk is a unique fluid which contains all the nutrients required by the newborn infant and a wealth of other bioactive components with non-nutritional properties that may promote infant health. In this paper, I shall describe the range of bioactive components that have been identified in human milk and discuss their likely mode of action, drawing on our cross-cultural studies in England and The Gambia for illustration. Finally, I shall provide a current perspective on the importance of breast-feeding to infant growth, health and development.

The composition of human milk

The nutrients of human milk provide the substrate materials required for tissue growth, development and maintenance, such as amino acids, fatty acids, minerals, vitamins, trace elements and water, and supply the energy for metabolic processes in the form of fat, protein and carbohydrate (1-3). The specific nutrient composition of human milk is ideally suited to the needs of the newborn infant. For example, human milk fat contains a comparatively high proportion of long-chain polyunsaturated fatty acids, such as arachidonic acid (20:4ω6) and docosahexaenoic acid (22:6ω3), which are important constituents of brain and neural tissue and are needed in early life for mental and visual development (4-6). The presence of ß-casein as a major protein component, and a high proportion of palmitic acid in the central position of triglyceride molecules, aid digestion and absorption (4, 7, 8). The low sodium content is suited to the renal handling capabilities of the neonate, and the low energy and protein density is compatible with the slow growth rate of the human infant (9, 10).

Human milk also contains a wide variety of biologically active components with properties that are unrelated to nutrition in the classical sense (1-3). These include antimicrobial and anti-inflammatory factors, cytokines, digestive enzymes, transport agents, hormones, trophic factors and growth modulators. Some examples are listed in Table 1.

There is no clear distinction between nutritional and non-nutritional components, or between different categories of biological activity. Nutrients, such as fats, proteins, carbohydrates and vitamins, can have additional non-nutritive properties, while non-nutritional components function as nutrient sources after digestion and absorption. Many substances have multiple roles and can have synergistic functions with other components. For example, lactoferrin, one of the major whey proteins of human milk, has bacteriostatic and bacteriocidal activities, is involved in the transport and absorption of iron, modulates cytokine production, has epidermal-growth promoting properties, enhances the hydrolytic activity of fat-digesting enzymes, and is

ultimately degraded in the infant's gastrointestinal tract, presumably releasing amino acids for absorption (11-16). Certain bioactive factors are generated during the digestion of milk components in the infant's gastrointestinal tract. For example, ß-casomorphins, opioid-like substances that may influence infant mood and behaviour, are produced by the degradation of casein, one of the main nutritional proteins (17, 18) and antimicrobial fatty acids and monoglycerides are produced from human milk fat by the action of bile-salt stimulated lipase (13, 19).

Table 1. Examples of human milk components with non-nutritional properties.

Antimicrobial factors	*Cytokines/anti-inflammatory*	*Hormones*
Secretory-IgA, IgM, IgG	Tumour necrosis factor	Feedback inhibitor (FIL)
Lactoferrin	Interleukins	Insulin, cortisol
Lysozyme	Interferon-γ	Prolactin
Fibronectin	Prostaglandins	Thyroid hormones
Leukocytes	α_1-antichymotrypsin	Corticosteroids, ACTH
Complement-C3	α_1-antitrypsin	Oxytocin
Lipids and fatty acids	PAF acetyl hydrolase	Calcitonin
Antiviral mucins, GAGs	Antioxidants	PTHrP
Oligosaccharides		Erythropoetin
Growth Factors	*Transporters*	*Digestive enzymes/others*
Epidermal (EGF)	Lactoferrin (Fe)	Amylase
Nerve (NGF)	Folate-binder	BSS-esterase, BSS-lipase
Insulin-like (IGF)	Cobalamin-binder	Lipoprotein lipase
Transforming (TGF)	IGF-binder	Casomorphin
Taurine	Thyroxine-binder	∂-sleep peptides
Polyamines	Corticosteroid-binder	Nucleotides, DNA, RNA

Although the concentrations of many of these bioactive components are low, several are present in surprisingly large amounts. Secretory-IgA and lactoferrin, for example are among the four most abundant proteins of human milk (together with casein and α-lactalbumin) and, in colostrum, secretory-IgA predominates (2). Similarly, oligosaccharides, principally lactose-N-tetraose and its monofucosylated derivatives, at a concentration of 3 - 6 g/L represent 5 - 10% of the carbohydrate fraction of human milk (20).

Table 2. Content of selected bioactive components in mature human and cow's milk (g/L).

	Human	Cow
Oligosaccharides	5	0.05
Lactoferrin	2	Trace
IgA	1	0.03
IgG	0.01	0.60
Lysozyme	0.50	Trace
Taurine	0.05	0.002
Amylase	Present	Trace
Bile-salt stimulated lipase	Present	Absent
Platelet-activating factor acetylhydrolase	Present	Absent

Data from (2, 21, 22)

The concentration of many of the bioactive components in human milk differs greatly from that found cow's milk, in addition to the many interspecies differences in nutrient composition (1, 2,

23). Some examples are given in Table 2. As can be seen, IgG is the main immunoglobulin of cow's milk rather than secretory-IgA, and lactoferrin is present only in trace amounts. These differences generally apply to formula milks, since these are largely based on cow's milk, although some brands do adjust the concentration of certain factors, such as taurine, to more closely resemble that found in human milk (23).

Functions of bioactive components

The biological activities of the non-nutritional components of breast-milk have been recognised largely from studies in animal models and in vitro systems and, in most instances, their physiological significance is poorly understood. The principal site of action may be in the alimentary canal and related mucosal surfaces of the infant, within the body of the child, or in the breast of the mother. It is likely that a number of substances represent 'spill-over' or excretory products from metabolic processes in the mammary secretory cell and have no further biological function once secreted into milk. The following short sketches illustrate the diversity of bioactive components and their possible functions in human milk.

Secretory-IgA

Secretory-IgA is the principal immunoglobulin of mucosal secretions in the human. It is a dimer consisting of two monomers of IgA coupled by secretory component and J-chain, a particularly stable configuration that is resistant to proteolysis and that can survive in the relatively hostile environment of mucosal surfaces. Although its concentration declines during lactation from the high levels present in colostrum and can be influenced by a number of factors including maternal parity, nutrition, season and geographical region (24, 25), the intake of secretory-IgA by breast-fed children is considerable (Table 3) (14, 15). Significant amounts of ingested secretory-IgA survive in the infant's gastrointestinal tract. Secretory-IgA is present in the faeces of breast-fed infants at levels that are many times greater than those found in the faeces of formula-fed or weaned children (Table 3) (14, 15). Calculations based on the relationship between faecal output and defaecation rate suggest that at least 30% of ingested secretory-IgA survives digestion in the stomach and small intestine, regardless of age and the co-consumption of solid foods (15).

Table 3. Breast-milk secretory IgA intake and faecal output in Gambian and English children

	Age (m)	N	Intake (mg/d)	Output (mg/d)	Output (%)[a]
Gambian					
Breast-fed	1.5	5	600 (50)	111 (17)	19 (3)
Breast-fed	3	10	530 (50)	28 (3)	5 (1)
Breast-fed	17	8	300 (80)	15 (8)	4 (3)
Fully weaned	34	7	-	3 (2)	-
English					
Breast-fed	1.5	10	947 (120)	160 (28)	17 (1)
Breast-fed	3	10	842 (98)	94 (17)	11 (1)
Non-breastfed	1.5	9	-	14 (2)	-
Non-breastfed	3	6	-	25 (5)	-

[a] output relative to intake. Faecal outputs of breast-fed children were significantly greater than those of non-breastfed in the same community. Data from Prentice et al (14, 15).

Young infants have immature defence systems and the production of secretory-IgA in their mucosal secretions is low. Supply of maternal secretory-IgA via human milk may compensate for this underdeveloped mucosal defence both in the gastrointestinal tract and, after aspiration, in

the respiratory tract. Human milk secretory-IgA has antibody activity to a range of enteric and respiratory pathogens (3), including bacteria (eg *Escherichia coli)*, viruses (eg *Haemophilus influenzae*), parasites (eg *Gardia lamblia*), and fungi (eg *Candida albicans*). The antibody specificity of human milk IgA reflects maternal exposure to mucosal infection and does not mirror the antibody profile in blood (26). Breast-fed children are thus furnished with secretory-IgA antibodies against the common mucosal pathogens in their own environment to which they might be exposed.

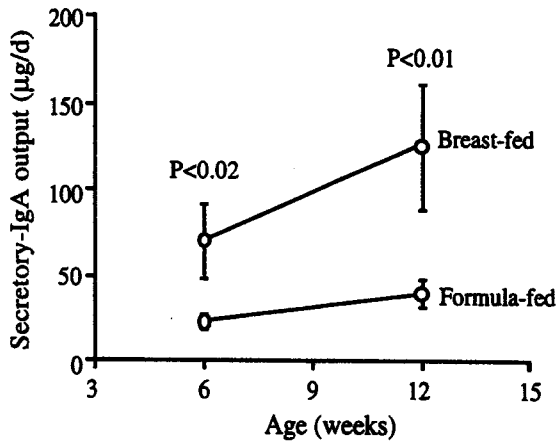

Figure 1.
Urinary output of secretory-IgA in breast-fed and formula-fed English infants.

Results from 10 breast-fed and 12 formula-fed infants studied on two occasions.

From Prentice 1987 (27).

Secretory-IgA is present in the urine of breast-fed infants at higher concentrations than in formula-fed infants of the same age (Figure 1) (27, 28). Since the size of the secretory-IgA molecule is too large to be filtered by the kidneys, this is unlikely to reflect absorption and excretion of ingested human milk protein, and suggests that breast-feeding promotes the development of the infant's immune system. Breast-fed infants also have enhanced immune responses to vaccination with diphtheria and tetanus toxiods, oral poliovirus and Haemophilus influenzae type b polysaccharide, and to infection with respiratory syncytial virus (16). The mechanism of immunostimulation is not known, but may involve anti-idiopathic antibodies (anti-antibodies), leukocytes, nucleotides or other immunostimulants present in human milk (16).

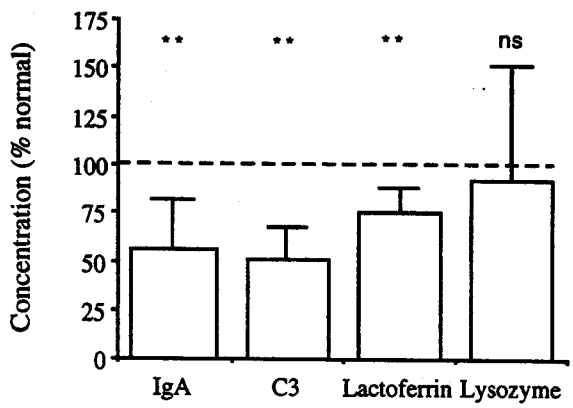

Figure 2.
Antimicrobial factors in non-mastitic milk of Gambian women with a history of mastitis.

The concentration of IgA, complement-C3, lactoferrin and lysozyme in the non-mastitic breast-milk of Gambian women with a history of mastitis (n=10) compared with other mothers in the same community (n=152), data are mean concentration in percent (SE). ** P<0.001.

From Prentice et al 1985 (29).

Secretory-IgA and other antimicrobial factors in human milk, in addition to the putative antimicrobial role in the gastrointestinal and respiratory tracts of the infant, may also be important in protecting the lactating breast from infection. Micro-organisms, such as *Staphyloccocus aureus* enter the breast via the nipple and cause mastitis when milk stasis provides a conducive environment for bacterial multiplication and tissue invasion. Antimicrobial factors in milk bathing the epithelial surfaces of the breast may reduce the likelihood of infection, with obvious benefits for the mother and the nursing infant. This hypothesis has been little explored but is supported by a study in The Gambia which demonstrated that women with a history of mastitis had lower concentrations of IgA, lactoferrin and complement-C3, but not lysozyme, in their non-mastitic milk than other women in the same community (Figure 2) (29).

Amylase and lipase

The digestive system is immature at birth and develops slowly during early life. The secretion of pancreatic and salivary amylase, required for starch digestion, is low in the newborn and does not reach adult levels until 1-2 years of age (30-32). Similarly, young infants have low secretion of bile acids and pancreatic lipase, required for fat digestion (31, 33, 34). In addition, undernourished infants have reduced levels of many components of the digestive system, including bile salts and digestive enzymes (35-37). This is illustrated for salivary amylase in Figure 3 for infants in The Gambia where growth faltering is common.

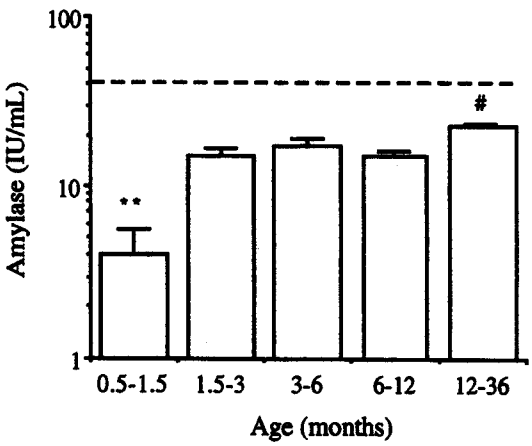

Figure 3.
Salivary amylase activity in Gambian children.

Amylase activity measured by maltotetraose hydrolysis at 37°C in 101 Gambian children aged 0.5-36 months. Data are mean (SE), on a logarithmic scale. Dotted line is mean value for 11 English infants aged 11-36 months.
P<0.001 for comparison between Gambian infants aged 0.5-1.5 m and older children.
# P<0.05 for comparison between Gambian and English children aged 12-36 months.

From Prentice et al 1991 (37).

Human milk contains digestive enzymes that are particularly suited to the conditions prevalent in the gastrointestinal tract of the young infant and may contribute to the digestion of the milk itself and of weaning foods. Human milk α-amylase (EC 3.2.1.1) has a broad pH optimum range of 4.5 - 7.5, is relatively resistant to acid conditions and peptic degradation, and remains active in the infant's small intestine (22). It is plausible, therefore, that this enzyme may promote the gastric and intestinal digestion of starch in foods co-ingested with human milk (38). Human milk bile-salt stimulated lipase (EC.3.1.1.3; BSSL), known by a variety of names including milk digestive lipase and bile-salt stimulated esterase, has wide substrate specificity and will hydolyse both emulsions and water-soluble substrates (19, 22). BSSL is stable in acid conditions, survives transit through the stomach, remains active in the infant's gastrointestinal tract, and, unlike pancreatic lipase, can function at low bile-salt concentrations (39). The products of fat hydrolysis by BSSL include fatty acids and glycerol. These are more readily absorbed than the hydrolytic products of pancreatic lipase (19), and several have antimicrobial properties (40, 41). BSSL may also be important in the digestion of retinyl esters, the main source of vitamin A in human milk (19, 39).

Human milk contains appreciable activities of amylase and BSSL throughout lactation (22, 42, 43). Colostrum and early milk are richer in amylase, but lower in BSSL, than mature milk (22). There are marked, consistent differences in enzyme activities between milks from different mothers, which result in a wide range of daily intakes by the infants (22, 42, 43). The differences in amylase activities and intakes are evident in Table 4 which gives measured values for four typical Gambian mothers and their babies. There is evidence that the activities of these enzymes may be reduced by maternal malnutrition and high parity (22, 42).

Table 4. Activity and intake of breast-milk amylase in 4 Gambian mothers and their infants

	Amylase activity (IU/mL)	Breast-milk intake (mL/d)	Amylase intake (IU/d)
Subject A	2.59	524	1357
Subject B	1.34	832	1115
Subject C	0.74	931	689
Subject D	0.11	829	91

Amylase activity was measured by hydrolysis of maltotetraose at 37°C; breast-milk intake by deuterium oxide dilution. The infants were 1-3 months old. Data from Dewit et al (42).

The importance of human milk enzymes to digestion and absorption by breast-fed infants is not established. There is evidence that BSSL improves fat absorption in the neonate, especially those born prematurely (44, 45). However, recent studies in our laboratory using stable-isotope labelled foods have not been able to demonstrate enhanced fat and starch digestion among older Gambian infants as a result of co-consumption of breast-milk (46, 47).

Feedback inhibitor of lactation (FIL)

Breast-milk output varies to match the demands of the nursing infant (48-50). The frequency and completeness of milk removal are important local signals in the regulation of milk production, independent of systemic galactopoetic hormones (51). This has been elegantly demonstrated by experiments in goats, which showed that an increased frequency of milking, from twice to three times daily, in only one udder produced increased milk yields in that gland only (52). The local, rather than systemic nature of this control, has also been illustrated in humans (49, 50) and is supported by the observation that the daily milk output of women with one dysfunctioning breast is similar to that of women with two healthy breasts (53).

Recent studies have pointed to the involvement of an autocrine factor in the local control of milk production (51). Initially identified in caprine milk, the feedback inhibitor of lactation (FIL) has also been detected in human milk (54). FIL is a small, acidic whey protein, molecular mass 6-30 kD, that inhibits lactose and casein synthesis in a rabbit explant bioassay. The mechanism of autocrine regulation is not fully understood, but studies in goats suggests that FIL is continuously secreted into milk and accumulates in the alveolar lumen between feeds (51). The increased luminal concentration of FIL inhibits milk synthesis in the secretory cell. In this way, FIL produces a decrease in milk synthesis rate as the gland fills and its removal with milk during nursing stimulates milk production (51).

Health benefits of breast-feeding

There is compelling evidence that breast-feeding protects the young infant against infectious diseases (3, 55). The effect is particularly strong for children from poor, underprivileged families in the developing world, but there is increasing evidence that breast-feeding reduces the incidence of gastrointestinal and respiratory infections even in affluent communities (3, 56-58).

This protective effect diminishes as the child grows older and after the introduction of solid foods or other milks (56, 58). However, in poorly nourished children, continued partial breast-feeding reduces the severity of infections and the risk of dying even in older infants and toddlers (55, 59). The effect of breast-feeding on maternal fertility and birth spacing, is also a major factor in reducing infant mortality in developing countries (60).

Benefits for child health are also evident in children many years after breast-feeding has stopped. Advantages, in terms of mental development and reduced risk of chronic childhood diseases, such as Crohn's disease, lymphoma, allergic disease and juvenile-onset diabetes mellitus, have been reported for older children who were breast-fed in infancy (3, 61-65). Investigations of the impact of early life nutrition on adult degenerative diseases indicate a reduced risk of death from ischaemic heart disease in men who were breast-fed in infancy except for those still being breast-fed at one year (66). This effect may be mediated by effects on infant weight gain, and alterations in cholesterol metabolism (66-69).

It is not clear whether the advantages of breast-feeding are a direct result of the ingestion of human milk, with its unique mix of nutritional and non-nutritional components, or are a reflection of the many social factors that affect a mother's decision to breast-feed, such as educational attainment, socioeconomic standing, housing and family background. It is also possible that breast-feeding may act passively by reducing exposure at an early age to certain substances present in alternative forms of nutrition (3, 70). Studies seeking to evaluate the consequences of breast-feeding are highly prone to confounding (71), and it is, therefore, virtually impossible to disentangle the multitude of interacting factors in order to determine the importance of individual bioactive components of human milk to infant health. However, the current evidence is that, taken together, there are major benefits in encouraging all mothers to exclusively breast-feed for the first 3-6 months of an infant's life, followed by the gradual introduction of solid foods (55, 72). In addition, there are many advantages of continuing breast-feeding for 1-2 years, as part of a mixed diet, particularly for mothers living in poor environments (55).

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