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Human Milk beyond Nutrition Role to Functional and Adaptative Physiological Functions

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Abstract

Human milk contains a large amount of various proteins that contribute to the newborn's quality of life. These proteins, when digested, are transformed into peptides and amino acids that help in better absorption of nutrients from milk, some of them such as; amylase, β-casein, lactoferrin, among others. They also have several activities, such as; immunomodulators, antihypertensives, antimicrobials, antithrombotics and others.

Human milk can be divided into three phases; colostrum, transitional milk and mature milk and in these three different phases of milk the composition of proteins, peptides, amino acids, sugars and all other components fluctuate a lot.

Many of the proteins are synthesized in the mammary gland with some exceptions such as serum albumin which comes from the blood circulation.

Currently, due to the great diversity and quantity of proteins and peptides in human milk, there are large studies on their properties such as; function and quantification of human milk proteins and peptides. With a view to better nutrition for newborns, better identification and biochemical characterization of these compounds. To this end, several steps will be carried out to isolate and characterize new peptides and proteins from human milk and the activities of the new peptides and proteins found in human milk will also be tested. These activity tests will be carried out in cell culture (cell proliferation or cell inhibition effect), blood pressure in rats (hypotensive or hypertensive effects) and guinea pig ileum (contractile or relaxing effect).

Once a peptide or protein with significant function or activity is isolated, peptidomic or cryptic analysis can then be carried out. New insights obtained with proteomics and metabolomics approaches revealed new components present in human milk including cryptides that could be generated in certain conditions by the newborn microbiome Therefore, by better characterizing human milk and its components, there will be better and greater conditions for understanding the nutrition of newborns and premature babies and a better understanding of the physiological role played by these components in relation to the development and growth of the newborn.

Keywords: Human milk; Bioactive peptides; Nutrition; Newborn

Introduction

General aspects

Milk is a liquid nutritious compound produced by the mammary glands of female mammals to feed their young. It is made up of water, a main protein, casein, a specific fat, butyrin and a specific sugar, lactose. Being the complete food for human nutrition. Contains all the basic components for human development. From a chemical-physical point of view, milk consists of different components, fractions or phases [1,2].

Human milk is the best food a child can receive as it has been specially designed to meet the needs of its species. What makes it unique is the fact that it satisfies the "nutritional-bond-stimulation-immunity" aspects, all these urgent needs of the newborn. No substitute food can satisfy these needs as completely as breast milk [3,4].

Breast milk is essential for the health of children in the first six months of life, as it is a complete food, providing components for hydration (water) and development and protection factors such as antibodies, leukocytes (white blood cells), macrophages, laxatives, lipase, lysozymes, fibronectins, fatty acids, γ-interferon, neutrophils, bifidus factor and others against common childhood infections, free from contamination and perfectly adapted to the child's metabolism.

Its nutrient content is adequate for the immaturity of the baby's kidney and intestinal function, for the growth and maturation of its brain and as raw material for the transformations that its body undergoes throughout the first year of life. These components are in such a proportion that none of them interferes with the absorption of the other. The supply of anti-infectious substances called immunoglobulins is the ideal complement for the baby's immune deficiencies. The chemical form in which iron and zinc are found is the ideal form for their best use [5].

There is also a special type of carbohydrate in breast milk that is necessary for the formation of a protective intestinal flora that inhibits the development of germs and intestinal parasites [6].

In relation to physical contact with the mother, it contributes to strengthening the psycho-affective bond, among other advantages such as:

- In comparison to the period of infertility, mothers who breastfed have longer periods of infertility than those who have never breastfed;
- Right after birth, breastfeeding stimulates the contraction of the uterus so that it returns to its shape more quickly;
- Most natural way to regain weight after pregnancy;
- Decreased risk of breast cancer.

Children who are not breastfed are at greater risk of acquiring a wide range of diseases such as: Diarrhea, eczema, colic, acute respiratory infection, otitis media, bacteremia and some types of meningitis. There is also a protective effect of breast milk against diseases. Late, such as: Asthma, type 1 diabetes and autoimmune diseases. Furthermore, infants who are fed cow's milk are more exposed to:

- Dehydrations
- Low levels of calcium as the excess phosphorus in cow's milk makes it difficult to absorb calcium
- Diarrhea
- Anemia, as the iron in cow's milk is not absorbed as efficiently as breast milk
- Ammoniacal dermatitis (diaper dermatitis), as the excess proteins in cow's milk are eliminated in the urine in the form of ammonia.

Review of Literature

In the first days of life, before the arrival of the final milk, the newborn will feed on colostrum, a dense, yellowish milk, called “first milk”. It is a milk full of nutritious substances, rich in proteins and minerals, important for the first days of life. And in addition, colostrum will provide certain antibodies that are defense substances to protect against possible aggression from germs and viruses. Around the third or fourth day, the colostrum changes its appearance, becoming clearer and creamier. It is the transitional milk that is used to get the newborn used to the final milk that will follow. Gradually, proteins decrease and the sugar content, essential for the growth of brain tissues and fat content that are transformed into energy increases. Upon completion of 10 days, the mother's breast will finally produce “perfect milk”, more fluid and with a sweet taste [7,8].

Human milk provides around 70 Kcal/ 100 mL. Lipids provide 51% of the total energy in milk, carbohydrates 43% and protein 6%. Lipids, in addition to providing energy, also have important physiological and structural roles, in addition to being a vehicle for the entry of fat-soluble vitamins from milk.

Lactose is the predominant carbohydrate in milk, where it helps in the proliferation of Lactobacillus bifidus which, by inhibiting the growth of gram-negative microorganisms, prevents the appearance of intestinal infections [6].

Human milk contains the lowest protein content, with the highest content in colostrum the first secretion of the mammary gland. Milk proteins are divided into casein and whey proteins. The largest amount of proteins in cow's milk is in the form of casein (82%), while in mature human milk the casein content does not exceed 25% of the total proteins [9].

Casein is an important protein as a provider of free amino acids to the infant, in addition to calcium and phosphorus which are the constituents of its micelles. Whey proteins (lactoferrin, immunoglobulins) are essential for the protection of the newborn.

Most vitamins are present in adequate amounts in human milk. Although cow's milk contains some vitamins in greater quantities than breast milk, heating, exposure to light and air inactivates and destroys most of them.

The electrolyte content of cow's milk is three to four times higher than that of breast milk and combined with the high protein content, it can cause kidney overload which can lead to sodium retention, hyperosmolarity and increased sensation of thirst.

Iron is present in similar concentrations in human milk and cow's milk, but it is better available in the former. Lactoferrin, a protein that binds to iron in human milk, reduces the amount of free iron, inhibiting bacterial multiplication [10]. Human milk contains lactalbumin, lactoferrin, lysozyme, secretory IgA, with iron being better absorbed. Carnitine (Trimethylnolamine) whose function is to transfer free and long-chain fatty acids into the mitochondria for oxidation and also taurine, a sulfur amino acid, important in vision (retina). It has Polyunsaturated long-chain Fatty Acids (PUFAS), with omega 3 (arachidonic, docosahexanoic) playing an important role in the formation of the Central Nervous System (CNS) and retina (63% of the brain structure is made up of lipids). Some amino acids, specifically glycine and arginine, are contained in human milk in quantities far below the newborn's needs. However, there are evidence that the newborn is capable of adjusting the transfer of nitrogen from amino acids in excess to those in deficit. However, work reviewing the use of human milk protein supplementation in premature babies shows a short-term increase in weight gain, length and head growth. Urea levels were increased and suggest that investigations are needed to evaluate the effects of protein supplementation on long-term growth and neurodevelopment [11].

There are more than 20 enzymes in human milk, including lipase, amylase and protease, which are important for digestion. The lipase found in human milk is responsible for the absorption of 95% of fats, it is thermolabile, therefore prefer unprocessed milk (pasteurization). The nutritional and functional importance and the great diversity of proteins present in milk explain the high interest in studying the composition of this fraction **(Figure 1)**.

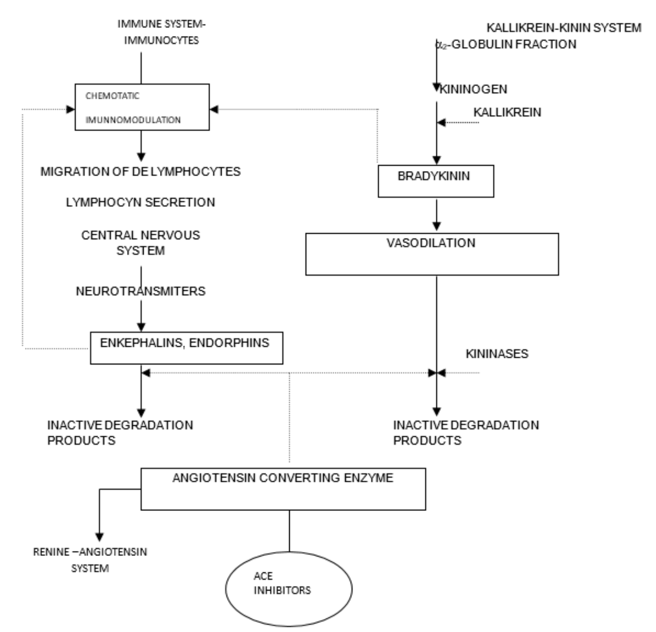

Figure 1: Different physiological systems pathways of the body influenced by peptides derived from milk such as modulation of blood pressure, immune response and hormone release by Angiotensin-Converting-Enzyme (ACE) (ACE inhibitors) central nervous system (casomorphins).

Bioactive proteins and peptides present in milk

The main proteins in milk (except serum albumin and immunoglobulins) are synthesized by epithelial cells in the mammary gland from amino acids extracted from the blood. The main proteins in milk include casein, β-lactoglobulin, α-lactalbumin and immunoglobulin.

Casein, β-lactoglobulin and α-lactalbumin account for 95% of milk proteins. The three possible origins of blood precursors of proteins synthesized by the mammary gland are:a

- Their concentration in blood plasma is lower than the amount necessary to supply 10% of the amino acids that form proteins synthesized in the mammary gland.
- Provide less than 10% of the protein synthesized in the mammary gland.
- Most of the nitrogen used for the synthesis of milk proteins comes from free amino acids absorbed by the mammary gland.

Recently Reinhardt, Lippolis and Smolenski et al. are carrying out proteomic analysis studies on milk samples, having detected up to 2903 spots corresponding to different proteins and peptide fragments, mainly those with immunological activity having been sequenced [12,13].

Proteins potentially generating bioactive peptides

The sequences bioactive they are found in inactive form, within the polypeptide chains of protein molecules, called peptide precursors. The biochemical effects of these peptides include activities ranging from the direct use of nutrients to actions on the individual's peripheral organs. Recent reviews by Lebrun, Pimenta and Lebrun specifically discuss the importance of sequences inserted into proteins, from those originating from proteins contained in foods to those that have evolutionarily demonstrated greater conservation with regard to the structure and function relationship as well as the identification of bioactive peptides based on the limited proteolysis of the "mother protein" **(Table 1)** [14,15].

Table 1: Location of immunostimulating peptides and β -casomorphins in the "strategic zones" of human and bovine β -casein.

	β -casein bovine	**β -casein human**
"Strategic zone"	^{60}YPFPGPIPNSL^{70}	^{51}YPFVEPIPYGFL^{63}
Immunostimulating peptides		
Hexapeptide	PGPIPN	VEPIPY
Tripeptide		GFL
β -casomorphins		
1 → 4	YPFP	YPFV
	YPFP.CONH$_2$ (morficetin)	YPFV.CONH$_2$ (valmucetin)
		YPDFV.CONH$_2$ (devalmucetin)
1 → 5	YPFPG	YPFVE
1 → 7	YPFPGPI	YPFVEPI
1 → 8	YPFPGPIP	YPFVEPIP

Albumin: Protein of high biological value, it is highly digestive and contains all essential amino acids in ideal quantities and proportions, it is essential for muscle growth and regeneration, in addition to helping with the transport of iron and binding to fatty acids, as well as other small molecules [16].

Immunoglobulin: In colostrum, these immunoglobulins are very concentrated, but in milk they are in low concentration. Immunoglobulins include IgG1, IgG2, IgA and IgM, they are part of the passive immunity transported to the neonate *via* colostrum in many species, but not in humans. They are part of the mammary immune system. From immunoglobulins it is possible to generate other protein -peptide components that may have relevant activity [17].

Exogenous proteins

There are several exogenous proteins in human milk, some of them are:

Casein: Found in milk in the form of micelles (dense protein granules), being a relatively hydrophobic phosphoprotein. It can be subdivided into polypeptide chains, which vary from 19 to 25 kDa, the most abundant of which are a α_{s1} with 119 amino acids, α_{s2} with 207 amino acids, β with 209 amino acids e casein with 169 amino acids. Micelles are composed of α , β e κ-casein. The α-casein has different multiphosphorylated forms (α_{s2}, α_{s3}, α_{s4}, α_{s5}, α_{s6}), whereas β-casein is the largest casein in bovine milk, but the smallest in human milk and κ-casein is distributed throughout the casein micelle and acts to stabilize it. There is also γ-casein which is made up of fragments of -casein that are released by plasmin digestion while the milk is in the mammary gland. This casein micelle serves as a source of nutrients for the newborn, providing highly digestible amino acids, calcium and phosphate [18].

Human casein represents only 30%-35% of the total proteins in human milk and is subdivided into β-casein, κ-casein e α_{s1} -casein. The α_{s1} chain of human casein is highly homologous in its N-terminal portion when compared to the α_{s1} chain of other species, such as bovine. Human β-casein has 47% homology with bovine β-casein and human β-casein has 22% homology with the bovine κ -casein chain [19].

Casein is a rich source of biologically active peptides, which can act as potent physiological modulators of metabolism. These peptides, present in their inactive forms within the casein sequence, can be released by enzymatic digestion, such as during gastrointestinal digestion or during food processing, therefore assuming their probable biological activities [18].

Many authors have isolated and described several peptides isolated from casein, which have diverse biological activities.

Discussion

Bioactive components of human milk

Human milk contains several substances with physiological functionality, one of which is the prevention of various infections. Most of these activities are attributed to antibodies, but a minority of proteins such as lactoferrin and lactoperoxidase and the milk sugar part are now being studied as bioactive agents **(Table 2)** [20,21].

Table 2: Comparison between human and bovine main active compounds present in milk [22-24].

Concentration (g/l)			
Proteins	**Bovine**	**Human**	**Function**
Total casein	26-0	2-7	Ions transporter, precursor foc bioactive peptides
α-casein	13-0	-	-
β-casein	9-3	-	-
κ-casein	3-3	-	-
Total protein whey	6-3	67-3	-
β-lactoglobulin	3.2	-	Retinol transporter, fatty acids binder, possible antioxidant

Ŭ-lactoalbumin	1-2	1-9	Mammary gland synthesis, Ca transporter, imunomodulador, anticarcinogenic.
Immunoglobulins (A, M, e, G)	0-7	1-3	Immunogical protection
Serum albumin	0-4	0-4	-
Lactoferrin	0-1	1-5	Antimicrobian, antioxidant, Immunomodulador, Fe absorpti -on, Anticarcinogenic
Lactoperoxidase	0-03	-	Antimicrobian
Lisozime	0-0004	0--1	Antimicrobian, sinergic effect with immunoglobulins and lactoferrin
Miscellaneous	0-8	1-1	-
Proteose-peptone	1-2	-	Not characterized
Glicomacropeptide 1-2	1-2	-	Antiviral, bifidogenic

Main activities obtained from peptides bioactives derived from milk proteins

There is peptide studies bioactives derived from milk protein, where has already been characterized activity of some of them. It could then be distributed according to your activity.

Antihypertensive activity

In studies on milk, peptides were observed that have an antihypertensive effect, which inhibits the angiotensin I-converting enzyme. The angiotensin I-converting enzyme plays an important role in regulating blood pressure, as it is responsible for the production of vasoconstrictor angiotensin II and inactivation of vasodilator bradykinin. The first ACE-competitive inhibitors that were described were snake venom peptides. Ferreira et al. isolated 9 bioactive peptides from *Bothrops jararaca* [25].

These peptides can be obtained through enzymatic hydrolysis while they are being digested or during food. While the structure of the activity related to ACE inhibition has not been established, it is believed that there is a very strong influence of the sequence C-terminal tripeptide [26].

Casokinins or casein-derived ACE inhibitors represent different fragments of bovine and human casein. For example, the high activity of casokinins is in bovine milk in sequence 23-27 of α_{s1} -casein (Phe-Phe-Val-Ala-Pro) and in sequence 177-183 of β-casein (Ala-Val-Pro-Tyr-Pro-Gln-Arg) [27].

Most casokinins have proline, lysine or arginine at its C-terminus opioid fragment of β-casormorphin 7 reveals a low ACE inhibitory activity [28]. Recently the sequence opioids correspond to β-lactorphin and reported dipeptides that have been identified with moderate ACE inhibitory activity [29].

Immunostimulating activities

Casein-derived immunopeptides include fragments of α_{s1} -casein (residues 194-199; Thr-Thr-Met-Pro-Leu-Trp) and β-casein (residues 63-68; Pro-Gly-Pro-Ile-Pro-Asn and 191-193; Leu-Leu-Tyr) [30].

The immunohexapeptide derivative of β-casein represents the C-terminal part of β-casomorphin 11. The sequence of the immunopeptide in ®human -casein corresponds to residues 54-59 [31].

Recently, Kayser and Meisel described the immunoactivity of human Peripheral Blood Lymphocytes (PBL) that were stimulated or suppressed by various bioactive peptides derived from milk proteins [32].

Tyr-Gly and Tyr-Gly-Gly peptides correspond to fragments of α-bovine lactalbumin and κ-casein, respectively, having significant activity in PBL proliferation. Depending on the concentration of the peptide, β-casokinin 10 and β-casomorphin 7, suppression as well as stimulation of lymphocyte proliferation is observed [33].

The mechanism by which peptides derived from milk proteins act as immunomodulators has not yet been defined, but some studies suggest that opioid peptides can affect the immunoreactivity of lymphocytes *via* the opioid receptor. This relationship between opioid peptides and the immune system is because endorphin opioid receptors present T lymphocytes and human phagocytic leukocytes [34].

Antimicrobial effect

Antimicrobial peptides derived from the whey protein lactoferrin, which is an iron-binding glycoprotein present in most fluids including milk. It is considered an important component for defense against microbial infections [35].

The 17-41 fragment of the peptide has an intramolecular bridge disulfide that is generated by enzymatic cleavage of lactoferrin with pepsin. This peptide, called lactoferricin, has bactericidal properties, a bactericidal agent was found in a fragment of $\alpha_{s2\text{–casein}}$ (position 165-203) [36,37]. This antibiotic peptide was named casocidin I, which inhibits the growth of *Escherichia coli* and *Staphylococcus carnosus* [38].

Antithrombotic effect

Casoplatelins are peptides derived from the C-terminal portion of bovine κ-casein, they are inhibitors of ADP aggregation -platelet activator as well as the binding of fibrinogen with the γ chain, specific receptor for platelets [39].

The main antithrombotic peptide is -casein in the sequence 106-116 (Met-Ala-Ile-Pro-Pro-Lys-Lys-Asn-Gln-Asp-Lys) and a small fragment 106-112, 112-116, 113-116 [40].

Opioid activities

The first bioactive peptides studied with opioid activity were from casein and were divided into three main groups: ®casomorphins, exorphins and casoxins.

A β-casomorphin corresponds to residues 60-70 of ®bovine casein and the sequence that demonstrated the greatest activity corresponds to the pentapeptide YPFPG and in β-human casein, PG residues are replaced by VE.

Opioid activity, the peptides must have the sequence YXF or YX_1X_2F or YXX.

Brant et al., in 1979, isolated a group of β-casomorphins that showed opiate activity on the longitudinal muscle of the mesenteric plexus of guinea pigs.

Such peptides have been shown to play a role in regulating appetite by modifying the endocrine activity of the pancreas, affecting intestinal motility and behavior [41].

Exorphins are peptides of exogenous origin, have activity similar to that of morphine and correspond to residues 90-96 of 〈 $_{s1}$-bovine casein and residues 41-44 of β-human casein, in this case, they are called β-casorphins.

Chiba et al. in 1989, isolated others, called casoxins, which are derivatives of -casein, showing opioid antagonist activity in tests with guinea pig ileum [42].

Other activities

In addition to the biologically active peptides mentioned so far, others have already been isolated and sequenced with specific biological activities. Among them inhibitory peptides, emulsifying peptides, bitter peptides **(Figure 2)** [19,43-50].

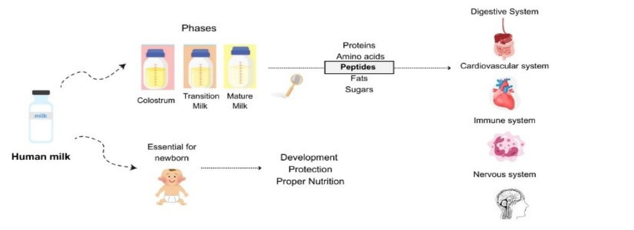

Figure 2: Graphical summary of main activities of peptides from human milk on physiological systems.

Conclusion

Instead human milk has been extensively studied and new insights have been presented recently using proteomic and metabolomic approaches revealing new compounds present in human milk, some of them namely cryptides a new category of peptides derived from a protein with new activities. Considering the newborn microbiome and light acid digestion the generated peptides as happens with the antibodies in the passive immunity could easily pass to the intestinal cellular membranes reaching the bloodstream helping not only in nutrition but also presenting other functions. All these data presented about milk composition and its nutritional importance, including the presence of proteins, peptides, enzymes and many other components; some questions remain unanswered. All these components have only nutritional role or they are fundamental components to adaptative process of the newborn in the first three month of living. Are these components a subset to the physiological systems helping them to reach the optimum physiological function? Or are these components the remaining of evolutive processes having now only secondary functions or a second pathway for the constitutive systems? All these aspects requires new data and new studies to be answered.

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