REVIEW ARTICLE

Human Milk: Benefits, Composition and Evolution

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Abstract
Breastfeeding provides all the energy that the child needs in the form of nutrients in the first months of life. The components cover the nutritional needs in all stages, including colostrum and final or mature milk. It must also be taken into account that the composition of milk varies from one woman to another, between both breasts, between feedings and in the different stages in the same mother. It can be said that variation is an active mechanism to perfectly adjust to the nutritional and immunological needs of each child. Components of breast milk can exert beneficial non-nutritional functions. Breast milk also has bioactive factors, which affect biological processes and, therefore, have an impact on health. In the nutrition of premature babies, parenteral nutrition is carried out first, which later becomes enteral through different strategies, such as early minimal enteral nutrition. Despite this, they still present postnatal growth restrictions, which is associated with adverse neurocognitive outcomes. Breast milk achieves multiple benefits in both preterm and term births. Digestion and absorption in the stomach and intestines follow circadian rhythms in mammals, and these rhythms are regulated by rhythmically expressed clock genes in the intestine, as well as by daily food intake.

Key word: human milk, lactation, newborn

Introduction

Breastfeeding is an evolutionary process that has ocurred over millions of years. There is evidence that suggests that primitive mammary glands would have developed in terrestrial animals ancestors of mammals, which carried out the gestation of their young in amnion (1, 2). In the evolutionary process, the integument of the pockets where primitive mammals incubated their eggs was defined into hair follicles and sebaceous glands that emptied into a kind of primitive nipples. Over time, the secretory glands developed other functions, eventually synthesizing a disaccharide, lactose. In mammals, the structures derived from the ectoderm were transformed into mammae in the territories called "milk lines" (3).

The complex evolutionary process led to the fact that mother's milk included, apart from proteins of high nutritional quality, nutrients that newborns required for proper development (4). One of the points that makes breastfeeding so recognized is the benefits both for the infant and for the mother, which are multiple and important in decision making regarding breast milk (4, 5, 6).

Breastfeeding constitutes the most solid foundations of newborns, it is the recommended diet par excellence, since it provides all the energy that the child needs in the form of nutrients in the first months of life (7). The Lactation Committee of the Spanish Association of Pediatrics (AEP) recommends breastfeeding for the first six months of life, crucial months for the infant's development (8). In addition, after the first six months, it is recommended to continue using breastmilk together with complementary foods that are formulated to fulfill children nutritional guidelines, since it continues to provide them with at least half of those nutritional needs during the second half of the first year, even reaching a third of those needs during the second year (7). Breast milk, as will be seen, has multiple benefits for both development and immunity, and its main power is to provide the newborn with the nutritional contributions it needs throughout the lactation period (9).

On the other hand, increasingly, perhaps due to the high level of stress to which we are subjected in our society, more premature children are born. These babies require hospitalization in the neonatal intensive care unit. As a consequence, your developing body needs to adapt metabolic and homeostatic pathways to the environment from this situation, increasing the risk of developing an adverse neurodevelopmental and metabolic outcome later in life (10).

Humoral and cellular immunity in breast milk

Breast milk contains antibodies against antigens from the environment. This fact can be explained by means of an anatomical reason: the presence of the so-called broncho-enteromamium link has been described in humans

The antigens penetrate the mucosa and reach the associated lymphoid tissue; it is in this lymphoid tissue where the antigen coexists with lymphocytes. These lymphocytes are then sensitized against ingested antigens and then travel to the mammary gland where they synthesize and secrete antibodies.

When the baby breastfeeds from the mother's breast, it acquires all those antibodies against the microorganisms to which its mother was exposed (11, 12). There are several studies that provide the idea that the cellular immunity provided by human milk is mediated by T lymphocytes and that it depends on cells that express positive CD3 + CD4 + or CD3 + CD8 + immunophenotypes. In addition, they conclude that the T cells present in the greatest quantity in human milk are those

with regulatory capacities, suggesting that these could be one of the first cell subtypes to colonize the organism of the recipient infant (13).

Composition of breast milk

The composition of breast milk is shown in Figure 3, although there are still gaps of knowledge regarding its components and ongoing research (14).

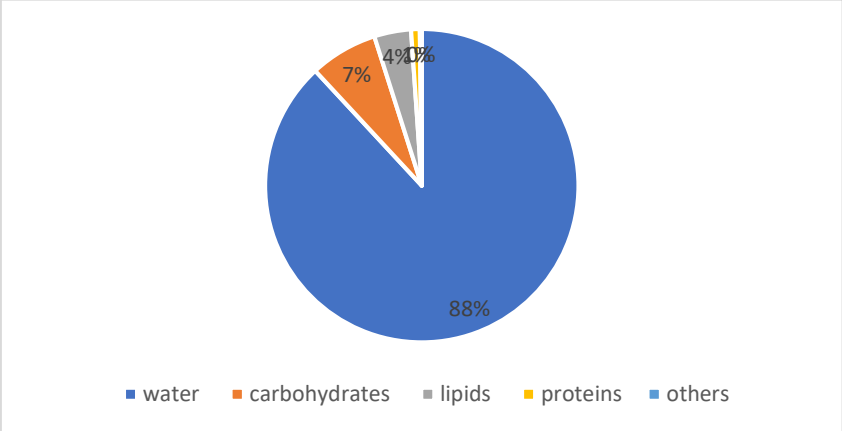

Figure 1: general composition of breast milk. Modified from Mandel et al. (14)

These components cover the nutritional needs at all stages, including colostrum and final or mature milk (14).

It must also be taken into account that the composition of milk varies from one woman to another, between both breasts, between feedings and in the different stages in the same mother. It can be said that variation is an active mechanism to perfectly adjust to the nutritional and immunological needs of each child. They are therefore functional changes (14, 15, 16). For example, the fact that the percentage of fat in milk increases significantly with the duration of lactation, have been verified during prolonged lactations, where the fat and energy content increased considerably. Something similar occurs when fat production is increased at the end of the feed, causing satiety in the infant (15).

In the case of colostrum, it is clear that the composition is richer in proteins, lactoferrin, immunoglobulin A, beta carotenes, vitamin E and B12, sodium and potassium, while a lower lactose content is observed. On the other hand, in lactations of more than one year, the composition of the milk becomes richer in fat and energy (15).

Components of Breast Milk with non-nutritional benefits

Components of breast milk can be found to exert beneficial non-nutritional functions, enhancing the idea of the suitability of breastfeeding as a baby food (17):

- Bifidobacteria: inhibit the growth of enteric pathogens.
- Inhibitors of the pathogenic metabolism of microbes: lactoferrin and the proteins that bind folates and vitamin B12 prevent the growth of germs (18, 19).
- Enzymes: lysozymes, peroxidases with bacteriostatic activity and other enzymes that act in the mammary transport and synthesis of the components of breast milk in the digestion and metabolism processes of newborns.
- Other anti-infective agents: factors that stimulate the proliferation of leukocyte colonies such as granulocytes and macrophages, or both, and fibronectin,

which facilitates the function of phagocytes.
- Immunoglobulins: fundamentally the secretory IgA with a higher concentration in colostrum than in definitive milk, which protects against germs from the mother's gastrointestinal tract. IgM, IgG, IgE, IgD, complement (C3 and C4).
- Immunomodulating agents: prolactin, secretory IgA, prostaglandin E 2 and some cytokines.
- Peptides: casein derivatives that regulate gastrointestinal motility and others such as the gastrin inhibitor peptide, which act on gastrointestinal growth, maturation and regulation.
- Lipids: certain medium-chain fatty acids and the monounsaturated and polyunsaturated ones exert an antiviral, antibacterial and antifungal activity.
- Growth factors: epidermal growth factor and insulin-like growth factors. Both favor the cellular proliferation of the intestinal epithelium and have anti-inflammatory activity.
- Somatostatin: with immunosuppressive and anti-inflammatory properties in the gastrointestinal tract of the infant.

This wide variety of substances provide the mother's milk with a broad spectrum of antiviral properties (20). Greater knowledge of these bioactive compounds could help identify new strategies to fight viral infections, even at a later age.

A recent study by Donalisio et al. (2020) investigated the antiviral activity of extracellular vesicles (EVs) extracted from the colostrum of mothers who gave birth prematurely. This study demonstrated an antiviral activity of colostrum against Cytomegalovirus (CMV) and an even more marked activity of the EVs in it, especially concerning some proteins present on their surface. This is particularly interesting when considering the risk of vertical transmission of CMV via breast milk in a susceptible population, such as in premature babies (21).

Kinds of milk

The mammary gland will secrete different types of milk: colostrum, preterm milk, mature milk and transitional milk.
- Colostrum: is the first milk of lactation, it is secreted within the first four days after delivery; it is yellowish and with a fairly high density. Its volume increases until the third day. It plays an important role in the transition from intrauterine to extrauterine nutrition. Its production is directly proportional to the intensity of the sucking stimulus (13). It is carried out when the junctions of the epithelium of the mammary gland are still open, so that the paracellular transport of substances is allowed and immunologically active from maternal circulation to milk (19).
- Transitional milk: is the milk that is produced between 4 and 15 days after giving birth. This milk is different every day since its composition changes to become mature milk, just as the production volume increases (22). An increase in the content of lactose, fat and water-soluble vitamins, with respect to colostrum, can be detected; a decrease in proteins, immunoglobulins and fat-soluble vitamins and in short, a substantial increase in calories can be observed. These changes occur abruptly and is stabilized around the fourteenth day; the volume production of the mammary gland is between 600 to 750 mL / day (23, 24).
- Mature milk: it is produced two weeks after delivery. Its composition includes not only proteins with high nutritional quality, but all the nutrients that newborns require for their proper growth and development (4). The perfect volume is achieved, which is around 700-900 ml / day. 90% of the volume is water and contains a high concentration of fat and high molecular weight proteins (23).

- Preterm milk: it is produced in preterm births. Milk is different and adapted to the needs of the child who has been born early and therefore will have important differences.

Each of the types of milk has properties that help the baby's development at different stages. Thus, breast milk the most appropriate food for the development of the baby.

Bioactive factors in breast milk

Breast milk also has bioactive factors, which affect biological processes and, therefore, have an impact on health. These bioactive components come from different sources (24):

• Produced by the mammary epithelium.
• Produced by carrier cells within milk.
• Others are extracted from the maternal serum and transported through the epithelium, by receptors.

Some of the biological factors studied are: macrophages, stem cells, inmunoglobulins (IgA, IgG, IgM), cytokines (IL-6,7,8,10, IFN-ganma, TGF-beta, TNF-alfa), chemokines (G-CSF, MIF), Citokine Inhibitors (TNFRI and II), Grow factors (EGF, HB-EGF, VEGF, NGF, IGF, Erythopoietin), Hormones (Calcitonin, Somatostatin), antimicrobial (lactoferrin, lactadherin/MGF E8), metabolic hormones (adiponectin, leptin, ghrelin, oligosaccharides and glicans, HMOS, gangliosides, glycosaminoglycans), Mucins (MUC1, MUC4) (24, 25).

By studying these components, differences amongst the stages of production have been discovered. The percentage of bioactive factors in term milks are very different with respect to the production of milk in premature infants (26).

Therefore, it is provided that when the use of donor milk is necessary, it must be fully adapted to the stage of development of the recipient baby. In addition, the most important thing is the preservation of that biological activity that milk has, through a process of collection, storage and adequate pasteurization.

The current research on bioactive factors, provides clear evidence that breast milk is not just nutrition as such. Breast milk contains factors that play a large role in the survival and health of babies. They represent the ability to respond to the changing needs of newborns (24).

The total knowledge of bioactive factors will lay the foundations for the development of new preventive approaches and new therapies based on the health of newborns (26).

Components of breast milk and health

As explained, the act of breastfeeding provides babies with multiple bioactive factors that will directly influence their health. Due to the direct action on health, numerous research projects are being carried out to make the most of the benefits that milk brings to children's health (24). One of the successful applications of the final milk, which is more energy dense due to its higher concentration in lipids, has been its use to to improve the growth of very premature babies. It has been also recommended for its nutritional management (25). In addition, by understanding the dynamic variability of human milk, donor milk banks have established protocols to “bundle” different milk donations to achieve a more uniform milk distribution (24). The mother's diet influences the content of DHA, so that through the supplementation of 1 g of this component, the values in breast milk increase significantly, thus improving the diet in babies (25). Another source of study in the modification of breast milk is the immunization of mothers. Immunization trials that are underway have shown increases in the level of

protective immunoglobulins in milk, thus having a direct effect on infants and improving immunity through breast milk (26). Therefore, some of the components of breast milk are being proposed as new medical agents to work in the prevention and treatment of diseases. The most studied have been lactoferrin, epidermal growth factor, erythropoietin and some oligosaccharides (24). Thanks to all this, it is known that some factors in milk can be modified through intake, in order to optimize the growth and health of babies.

Premature and maternal lactation

It is estimated that around 15 million babies are born at less than 37 weeks gestational age worldwide. Prematurity is the leading cause of neonatal mortality. The population of preterm infants have very different nutritional needs among them, depending on the degree of immaturity they present (27). The birth of a premature baby results in the cessation of the supply of nutrients, especially proteins, and produces a negative nitrogen balance. Several studies support that aggressive early nutrition can allow a positive nitrogen balance to be achieved, always safely and effectively (28). In the nutrition of premature babies, parenteral nutrition is carried out first, which later becomes enteral through different strategies, such as early minimal enteral nutrition. Despite this, they still present postnatal growth restrictions, which is associated with adverse neurocognitive outcomes (29). The benefits of providing breast milk to premature infants are numerous: benefits for the host defenses, fewer infections, decrease in diarrhea and urinary infections, decrease in otitis, neurodevelopment, improvement in cognitive development, improvement of visual function, protective effect against atopic disease, gastrointestinal effects such as gastric emptying and improvement of lactase activity (30).

Breast milk achieves multiple benefits in both preterm and term births. Breast-fed preterm infants were able to receive complete enteral feedings significantly earlier than preterm infants who received artificial preterm formulas (30). According to multiple studies, it is necessary to fortify human milk in order to meet energy requirements. Calcium, phosphorus, sodium, iron, and perhaps zinc need to be added (31). Breast milk is fortified to resolve insufficient nutrient intake and increase supplies. Several studies show that in neonates with very low birth weight the administration of unfortified human milk during hospitalization, and even after discharge, is related to growth failure and nutritional deficiencies. In addition, there have been studies in which weight and height were significantly higher in the statistics of infants fed fortified human milk for 12 weeks (32).

Breast milk today

Today, the storage of breast milk in our society is becoming more and more frequent, mainly due to the incompatibility with the moment of breastfeeding. But this process produces a loss in the nutrients and active components of breast milk, in different degrees according to the extraction method. As for example, it occurs with vitamin C and its sudden loss at the right moment of extraction, even giving the bottle at the same time (33).

The Academy of Breastfeeding Medicine has a protocol for the home storage of breast milk that can be used to guide mothers in these activities to optimize the integrity of expressed and stored milk (24). Many countries are also seeing the development of donor breast milk banks, including the exchange of milk over the internet. In order to control all this, a series of protocols are carried out consisting of

the pasteurization of donor milk with heating at high temperature and short time (HTST) (72 ° C for 15 seconds) and pasteurization of the support (62, 5 ° C for 30 minutes, a long time low temperature method [LTLT]); and flash heating (a low-tech HTST method that involves heating a jar of milk in a water bath that is quickly brought to a boil, then the milk is removed and rapidly cooled). All the methods are under study to be able to conserve the bioactive components and on the other hand eliminate the pathogens (34). Despite everything, the heat treatment of breast milk reduces the concentration and function of bioactive factors, especially with respect to proteins. Significant reductions after pasteurization have been demonstrated in IgA, lysozyme, BSSL, cytokines, lipases, TGF-β, and adiponectin, among other proteins (35). According to Garrido et al. (XX) when subjected to combined processing with compression and temperature, human milk improved the levels of compounds derived from lipid oxidation and the Maillard reaction such as ketones, aldehydes, pyrans and furans and alcohols. But this treatment decreases aliphatic hydrocarbons although carboxylic acids are not affected.

Much research remains to be done in this field, due to the advance in the use of these techniques and the increase in the use of breast milk expression before feeding by mothers.

Influence of maternal diet on milk composition

The composition of breast milk varies from one mother to another, the full reasons are still unknown, but they could be explained at first by diet and second by the environment. Therefore, a series of relationships and changes that are explained below: (36)

- The fatty acid profile is modified with the maternal diet, in such a way that the composition of the ingested fat is reflected in the milk fat (36).
- The main determinants of the fatty acid composition of breast milk are determined by the diet during pregnancy and the current diet during lactation.
- A diet rich in polyunsaturated fatty acids determines a higher content of these in milk (37).
- A diet with a predominance of carbohydrates over lipids will determine de novo synthesis of fatty acids in the gland, with a higher concentration of medium-chain saturated fatty acids (37).
- There is no evidence that cholesterol and phospholipids in human milk can be modified by the maternal diet.
- Maternal protein intake does not modify total protein levels (38).
- Lactose is the component with the greatest stability before the variation of the maternal diet.
- With regard to minerals, iodine and selenium are among those that are dependent on the maternal diet.
- Calcium, iron, zinc and copper would not be affected by the diet. But it has been observed that the dairy concentrations of iron, zinc and copper could vary depending on the geographical area (35).
- The concentration of vitamin A is in relation to the mother's diet and reserves, since the supplementation is not reflected in the milk content until the maternal deposits are covered (2).
- With respect to water-soluble vitamins, in general, a close relationship is observed between the concentration of thiamine, riboflavin, B6, B12 and C in breast milk and the mother's diet (2).

The proportion of the components changes depending on the different types of breast milk that develop in the different stages of lactation. Each of them is in optimal proportion for the correct development of

the baby. Also, the concentration of some nutrients in milk depends on the mother's diet. In most cases, the transport of nutrients to the milk will continue even in the case of maternal deficit. However, in some cases of inadequate maternal diet, the infant may experience inadequate levels of micronutrients, which can be critical for the infant's health. Vitamin K, as well as the concentrations of vitamin D in breast milk, were found to be insufficient, so they must be added to the baby's diet immediately after birth. Therefore, breast milk is not the only nutritional source for the baby at this age (39).

Chronobiology

Chronobiology is a word derived from three Greek terms: time (kronos), life (bio), and study (logos). It is the branch of physiology that studies the mechanisms of biological rhythms, their origin and their implications, along with biological processes that follow predictable temporal sequences (40). For centuries, efforts have been done to understand these daily cycles. It was the doctor and philosopher Galen who reflected in detail that vital processes had a rhythmic character. From that moment onwards, there were many studies and relationships that emerged around chronobiology, until it was consolidated as a scientific discipline after the 1960 international congress in Cold Spring Harbor, creating a scientific society for its study (40).

In this sense, our first nutrition, breastfeeding is involved in this. The mammalian circadian system is composed of a network of hierarchically organized structures that are responsible for the generation of circadian rhythms and their synchronization with the environment. This system is mainly formed by an endogenous timer, which is located in the suprachiasmatic nucleus (NSQ) of the hypothalamus, a brain structure located behind the eyes that detects the light signals that enter through the pupils (40). It was found that by destroying the suprachiasmatic nucleus, sleep-wake cycles and cortisone rhythms were lost. Since then, the NSQ is considered the central and main location of the circadian system in mammals (41). Under natural environmental conditions, the NSQ is "readjusted" every day by a periodic light / dark signal thanks to the existence of a non-visual pathway based on ganglion cells provided with the pigment melanopsin and in the retinohypothalamic tract. Although light (light / dark changes) is the main incoming signal to the NSQ, there are other periodic inputs, such as the timing of meals (intake / fasting) and scheduled exercise (activity / rest), capable of setting the time circadian system. The central pacemaker, in turn, synchronizes the activity of various peripheral clocks outside the NSQ through cyclical hormone secretion and the activity of the vegetative nervous system (42). To this day, it is known that the internal clock works thanks to the expression of several genes, like all functions in living beings, that can activate and deactivate it, showing a general 24-hour pattern, and that can be classified into positive elements (CLOCK and BMAL1) that activate the clock, and negative (PER and CRY) together with REV-ERB alpha (transcription factor) (43).

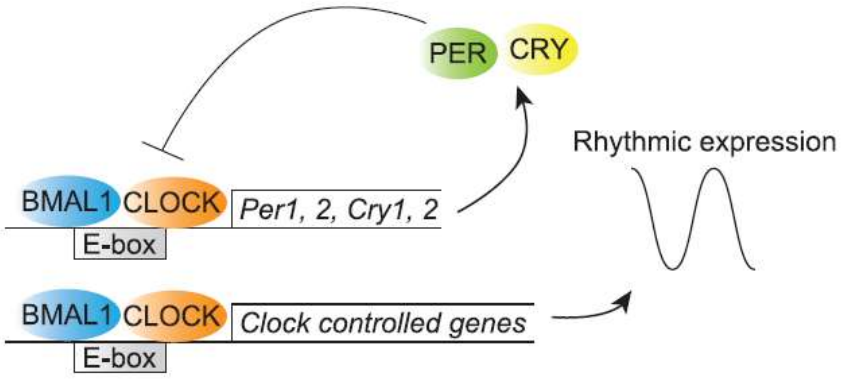

Figure 2: clock gen. Modified from Garaulet et al. (43)

Intracellular circadian clocks reside not only in the NSQ, but also in other peripheral tissues, including those involved in nutrient homeostasis, such as liver, muscle, and pancreas (43). The central clock synchronizes the activity of various peripheral clocks present in organs and tissues, such as the heart, lung, liver, oral mucosa, pancreas and adipose tissue, among others, through the cyclical secretion of hormones and activation of the autonomic nervous system. In order to carry out a schedule maintenance of all biological clocks, it is essential to maintain a rigid daily routine of rest, physical activity and food (40). A term of great importance is that of "Chronodisruption" which can be defined as an important disturbance of the internal temporal order of the physiological, biochemical and behavioral circadian rhythms. It could also be defined as the breakdown of synchronization between internal circadian rhythms and environmental 24-hour cycles. Several authors have indicated that chronodisruption may be an indicator of health in adults. In fact, a high fragmentation of circadian rhythms has been linked to the risk of mortality, brain disorders, cardiovascular diseases, cognitive decline, depression, drowsiness, aging and obesity (43). In addition, there are other physiological processes such as digestion, absorption and metabolism of food, closely related to energy intake and the effects of diet on the body, for which their circadian character is well known (42). The coordination of all tissue clocks contributes to a healthy state, despite the fact that each peripheral clock has its own physiological functions (for example, the brain clock for sleep or the liver clock for metabolism) (44).

Chrono-nutrition

The term "chrono-nutrition" has been used to refer to the relationship between food and the circadian clock system. We can change the timing of our internal clock by altering the timing of food intake. Chrono-nutrition attempts to address these two aspects: the timing of food intake or the contribution of food components to maintaining health, and the timing of food intake or the contribution of food components to rapid changes or readjustment of our internal clock system. Therefore, chrono-nutrition will be a common strategy to maintain our health through the circadian rhythm system (41). Physiological functions will have a circadian regulation. Digestion and absorption in the stomach and intestines follow circadian rhythms in mammals, and these rhythms are regulated by rhythmically expressed clock genes in the

intestine, as well as by daily food intake (45).

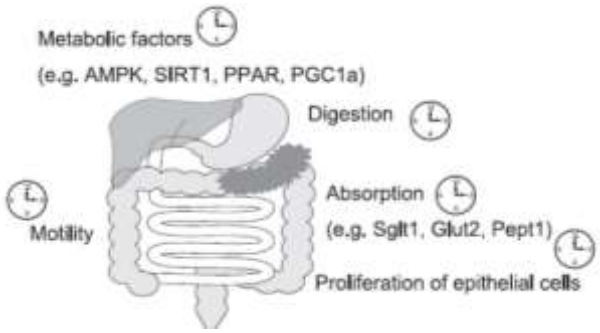

Figure 3: Chrononutrition. Modified from Garaulet et al. The image shows the different clock genes involved in digestion. (43)

Taking into account all the above, breastfeeding is recommended during the first years of life. In addition, it is recommended the milk expression times to be respected in the event that it is expressed beforehand. That is, the milk administration schedule must coincide with the extraction schedule to take full advantage of the benefits of this first superfood for newborns and during the first years of life.

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