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Review

HUMAN MILK IS NOT "MERELY NUTRITIOUS": HOW ITS BIOACTIVE ROLE CAN INFLUENCE CHILD HEALTH

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ABSTRACT

Maternal milk represents the best food for the newborn, providing specific physiologic advantages over the other sources of nutrition. It also contains many hundreds to thousands of bioactive molecules that protect against infection (i.e. IgA), inflammation (i.e. 5-methylthioadenosine) and contribute to eliciting strong immune responses in breastfed children (i.e. allergens or viral antigens). Quality and quantity of breast milk components may influence the development of infant body composition in the first years of life; in particular, it has been shown that a different composition of human oligosaccharides (HMOs) in overweight/obese women's human milk can be correlated with her offspring's growth. This could be associated with human milk's probiotic role, since probiotics support the assembly of a healthy gut microbiome, by stimulating the growth of beneficial microbes. Therefore, the aim of this review is to outline the bioactive role of human milk and its potential beneficial effect on a child's long-term health.

1. Introduction

Maternal milk represents the best food for the newborn, providing specific physiologic advantages over other sources of nutrition, because it provides the correct balance of macro and micronutrients for infant growth and development. Human milk has several bioactive compounds, such as antioxidants (i.e. melatonin), growth factors and hormones (i.e. Epidermal Growth Factor, EGF), adipokines (i.e. leptin, adiponectin), cytokines (especially the anti-inflammatory ones such as interleukin-10, IL-10) (1). Among these factors, some, such as human milk oligo-saccharides (HMOs) select for beneficial bacteria, and the specific human milk microbiota emerge as early mediators in the relationship between the development of gut microbiota in early life and short and long-term health outcomes (2). The influence that certain conditions such as the mode of delivery and the use of intrapartum antibiotic on breast milk bacterial composition is well known (3). The aim of this review is to highlight the importance of breast milk's bioactive role and how some bioactive factors present in human milk can have a potential beneficial action on the child's long-term health. In particular, we have focused our attention on cardiovascular, allergic and infection risk.

2. Human milk and non-communicable diseases: can milk composition influence their onset?

Role of breastfeeding mother's body mass index (BMI) in childhood obesity

The prevalence data show that obesity and diabetes mellitus are increasingly frequent in the pediatric population: a relatively low protein concentration in human milk, if compared with cow's milk, has a negative correlation with the development of these "non-communicable" diseases in adulthood. However, childhood obesity may be driven by differences in milk composition between lean and obese women suggesting that even a mother's milk could favor child obesity.

Milk from obese women has higher levels of bioactive compounds such as insulin, leptin, Tumor Necrosis Factor-α (TNF-α) and interleukin-6 (IL-6), when compared with a lean woman: quality and quantity of breast milk components may influence development of infant body composition in the first years of life.

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However, up to now, it has not been shown whether these substances do or do not play a pathogenic role in childhood obesity risk (4). An important role is performed by the anorexigenic hormone leptin: its concentration in human milk is almost twice as high in obese mothers than in normal-weight mothers at 1 month post-partum. (4) Leptin concentration in human milk during early lactation could influence infant body mass index (BMI), by interfering with hypothalamic activity during a critical period of early development (5). Nevertheless, it is likely that human milk composition is not the sole determinant of childhood obesity, but it can be one of many additive risk factors (4).Excess weight during pregnancy may influence the microbial composition of the gastrointestinal tract and the probability of becoming overweight/obese in childhood. The maternal gut microbioma may influence infant obesity risk through in utero programming effects or through vertical transfer of obesogenic gut microbiota from mother to child during birth and in breastmilk (6). Furthermore, high adenine concentration in the obese mothers' milk was associated with an earlier catch up growth in children at six months after discharge (4).

Proteomic and metabolomic patterns of human milk and maternal obesity

A recent study has established a discriminant breast milk proteomic pattern between obese mothers whose infants have delayed weight gain and normal-weight mothers whose infants have normal weight gain. It appears that obese mothers' offspring have a lower gain of weight in the first month of life with an early adiposity rebound in the first years of life and a higher probability to become obese (7). It is clear that a lot of biomarkers are implicated and one of these is a fragment of the sixth extracellular domain of the polymeric immunoglobulin receptor (pIgR), the over-expression of which in the mature breast milk of obese mothers, results in retarded growth and development of the newborn (7). Human milk has a higher abundance of nucleotides than cows milk and, in particular, 5-methylthioadenosine is positively associated with maternal BMI and with infant fat percentage at 1 month of life (4). 5-methylthioadenosine has a protective role against chronic inflammation and oxidative stress conditions characterizing obesity and metabolic syndrome. In this respect, its increase in the breast-milk of overweight/obese mothers could be protective for long-term weight gain, oxidative stress and cardio-metabolic risk (8). It has also been demonstrated that maternal obesity was associated, 6 months from delivery, with increased abundance of acylcarnitines, monosaccharides and sugar alcohols in breast milk, similar to individuals with obesity or type 2 diabetes. In particular, a relevant marker of glycemic control was 1,5 anhydroglucitol (AG) a reduction in which could predict the incidence of diabetes (4).

Relationship between human milk carbohydrates and lipids and body composition

A lot of studies have focused their attention on the role of human-milk oligosaccharides (HMOs) which can influence the body composition of children. However, despite there being no clear evidence available and a lot of contradictory studies, their concentration would appear to be positively associated with lean infant mass and negatively with adiposity. On the contrary, there would be no association with their daily intake.

It is likely that HMOs have an indirect effect on body composition of children, by enhancing the growth of gut bacteria and altering the gut microbiome during the critical period for the development of obesity (9).

A recent prospective observational cohort study has demonstrated that differences in HMOs composition could be part of the explanation for the early excessive weight gain in some exclusively breastfed infants. For example, an increase in lacto-N-fucopentose (LNFP) I was associated with lower infant weight at 1 and 6 months, instead LNFP II was associated with higher fat mass at 6 months (10). Also, the lipid component of the human milk can influence weight gain in childhood: a specific marker, called alkylglycerol-type (AKG-type), absent in infant formula and adult-type diets, maintains beige adipose tissue (BeAT) avoiding its transformation into lipid-storing white adipose tissue, using IL-6/STAT pattern. There would be a negative correlation between BeAT content and obesity: a premature loss of it, due to a lack of AKG intake, leads to a premature loss of this type of adipose tissue. Moreover, another mechanism of action of AKG is the increase of *Lactobacillus* proliferation with an establishment of a healthy intestinal microbiota (11).

However, even if a subset of metabolites seems to be correlated with both maternal and infant weight (Figure 1), the potential milk-dependent mechanisms for mother-child transmission of metabolic syndrome is still unclear.

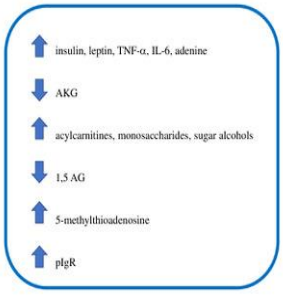

Figure 1. Characteristics of the human milk in overweight/obese women. (TNF-α: Tumor Necrosis Factor-α; IL-6: interleukin-6; AKG: alkylglycerol; 1,5 AG: 1,5 anhydroglucitol; pIgR: polymeric immunoglobulin receptor)

3. Breastfeeding and allergy risk: new perspectives

Even at the time of writing, the effects of exclusively breastfeeding on the development of allergic diseases in offspring is controversial: it can reduce eczema incidence in the first two years of life, can protect from *wheezing* and asthma development, but evidence in terms of food allergy is still unclear (12,13,14).

Recently, Ohsaki *et al* have demonstrated that the interactions of maternal food allergen-specific IgG immune complexes (IgG-IC) and neonatal crystallizable fragment receptor (FcRn) in induction of allergen-specific regulatory T (Treg) cell responses, are fundamental to the establishment of an effective long-lasting tolerance to foods. IgG-IC transferred during breastfeeding are necessary for optimal induction of tolerance (15).

Breast-feeding strategies and microbiota development

One of the most important factors associated to the microbial composition of human milk is the feeding strategy: numerous studies affirm that direct breast-feeding favors the acquisition of bacterial microbiota from the child's mouth, instead indirect breast-feeding (pumped-milk) increases environmental bacteria. This increased exposure to potential environmental pathogens is associated with a major risk of respiratory infections and increasing asthma in indirectly breast-fed children. Researchers from the University of Manitoba in Canada affirm that exclusively breastfed infants are more likely to produce milk containing "good" bacteria, on the contrary, indirect breast-feeding changes the baby's gut bacteria linked to asthma, such as *Stenotrophomonas* and *Pseudomonadaceae* (16). Klopp *et al*, including 2534 infants from the Canadian Healthy Infant Longitudinal Development (CHILD) study, showed that by 3 years of age, 12.6% of the children were diagnosed with possible or probable asthma, regardless of breastfeeding mode. Nevertheless, direct breastfeeding seems to be most protective against asthma development, while expressed breast milk appears to confer intermediate protection. This could be explained by two mechanisms: first, pumped-milk can be subjected by a degradation of bioactive components; second, infants who are directly breast-fed develop stronger lungs through the physical act of suckling in comparison with those who are indirectly breast-fed (17). Two main pathways have been proposed to explain the origin of milk microbiota: entero-mammary translocation of the maternal gut microbiota and retrograde inoculation by the infant's oral microbiota. Mother's obesity and diet can influence the first pattern, instead the mode of delivery and breastfeeding can alter the second one. It was demonstrated that *Bifidobacterium spp*., that are crucial in the development of the infant immune system, were prevalent in direct breastfeeding but depleted in indirect breastfeeding, with an increased risk of developing asthma in the latter group of children (16).

Exogenous antigens in breast milk

Recent studies in mice and humans have shown that the presence of foreign antigens in breast milk, such as allergens or viral antigens, could elicit strong immune responses in offspring who are breastfed. A recent study (18) shows that 15% of breast milk samples from mothers with asymptomatic malaria contain malaria antigens: Plasmodium falciparum Histidine-Rich Protein 2 (pHRP-2) and Plasmodium falciparum Lactate Dehydrogenase (pLDH). Levels of these antigens in human milk could strongly influence immune responses to malaria infection in breastfed children. At the same time, in a recent article, Baïz *et al* reported on the presence of Der p1 allergen in two/thirds of breast milk samples from French lactating mothers, with a higher concentration in the allergic ones. They observed an increased risk of allergic sensitization, asthma or allergic rhinitis at 5 years in children breastfed by mothers with Der p1 levels in milk above the median value compared with Der p1 levels in milk below the median value (19). Because the immune response depends on the levels (high or low) of antigen exposure, we can also confirm that oral exposure to house dust mite allergens from Der p1 could induce immunologic tolerance instead of sensitization, as has been observed in experimental models in mice, but further studies are needed (19). A recent randomized controlled trial (RCT) pointed out that increased maternal egg consumption is associated with more ovalbumin (OVA) in breast milk and increased serum IgG4 levels in infants, reflecting possible oral tolerance development in breastfed babies.

In line with the LEAP study (18) for peanuts, also for eggs, it would be helpful to introduce potential allergens early to promote the development of oral tolerance instead of food allergy (20). However, to fully confirm that OVA in breast milk is responsible for a decreased egg allergy risk in children, further RCTs will need to be conducted (21). In particular, it seems that the risk also depends on other cofactors, such as Transforming Growth Factor-b (TGF-b): OVA-immunized mice exposed to OVA during lactation had a minor risk of developing allergic diarrhea, especially if they were supplemented with TGF-b after weaning (22). At the same time, a vitamin A deficiency in neonates was the cause of their inefficient immune regulation, and a recent study has demonstrated that its supplementation inhibited eosinophilic airway inflammation and lung IL-13 secretion in mice who had received oral OVA administration during the first week of life (23).

4. Maternal milk and risk of infection

It is well-known that breast milk can sometimes be a vehicle of transmission of infection agents to the newborn and in the meantime also a source of specific bioactive immune molecules which play a role in reducing infection risk in breast-fed offspring (24). This protective role may be particularly strategic for newborns with additional risk factors for infection, such as prematurity, low birth weight, intrauterine growth restriction, congenital malformations, admission to an intensive care setting and colonization by multi drug resistant organisms (25,26,27).

HMOs, alkaline phosphatase (ALP) and IgA: a crucial role in necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is one of the most common and often fatal intestinal disorders in preterm infants. It has been shown that breast-feeding has a protective role especially in very low birth weight (VLBW) and in extremely low birth weight (ELBW) newborns. It is certain that HMOs protect against NEC in neonatal rats in fact, in a neonatal rat NEC model, pups were orally fed with formula without and with HMOs: the presence of disialyllacto-N-tetraose (DSLNT) improved survival and reduced NEC incidence, with a probable probiotic effect (28). In a multicenter clinical cohort study (29) a correlation was investigated between NEC (Bell stage 2 or 3) in VLBW children and disialyllacto-N-tetraose (DSLNT) concentration in human milk. The study revealed that DSLNT content in breast milk is a potential non-invasive marker to identify infants at risk of developing NEC. In particular, in almost all milk samples in NEC cases, DSLNT concentrations were significantly lower if compared with controls (29). There are a lot of pathways by which HMOs exert their protective effects on the gut, in particular they prevent the adherence of bacteria to the intestinal epithelium. In one study, it was demonstrated that an abundant HMO, found in breast milk – named 2'-fucosyllactose (2'FL)- is protective against an experimental mouse model of NEC. Perhaps this is due to the capacity of this oligosaccharide to increase intestinal perfusion up-regulating endothelial nitric oxide synthase (eNOS) pattern (30). A recent study has demonstrated that the intestinal metalloenzyme alkaline phosphatase (ALP) activity is reduced in premature infants as compared to early term or term ones.

These results are consistent with another study where premature rat pups have reduced ALP activity and expression compared to full term pups. This enzyme can reduce lipopolysaccharide (LPS)-mediated inflammation and could be an important protective factor in NEC development.

Human milk can be considered an important source of exogenous ALP, providing protection to the neonatal intestine against LPS-mediated inflammation during the critical window of bacterial colonization in the first days of life (31). Other bioactive components of maternal milk that can have effects on the neonatal microbiota are immunoglobulin A, present especially in *colostrum*. After their production by mammary glands, they undergo proteolytic cleavage and are released in the form of secretory IgA (sIgA). Breast milk sIgA provide an antimicrobial defense to the neonatal gastrointestinal tract by inhibiting pathogen attachment to mucosal surfaces and provisioning of passive immunity (32). Gopalakrishna *et al* (33) discovered that premature infants exclusively formula-fed, contained very low levels of IgA-associated intestinal bacteria and infants with NEC had higher levels of IgA-unbound *Enterobacteriaceae* when compared with healthy age-matched controls. So, we can assert that insufficient concentration of IgA and decreased IgA-bound bacteria in the intestine could be a risk factor for NEC development.

The role of oxysterols and of HMOs in Rotavirus gastrointestinal infection

Many factors contained in human milk stimulate the development of the immature system of the newborn and provide the infant with a first line of defense against pathogens through a passive transfer of acquired and innate immunological factors. In particular, some metabolites of cholesterol biosynthesis, known such as oxysterols, play many roles in inflammation and immunosuppression. 27-OHC is the most represented enzymatic oxysterol in human blood and the most produced in the mammary gland, especially in *colostrum*. Findings suggest that this oxysterol might contribute to the known protective activity of human milk against gastroenteritis caused by Rotavirus (34). In particular, even if not yet demonstrated directly, an intestinal absorption of 27-OHC in the intestine of breastfed infants appears very likely, and consequently, its protection against Rotavirus infections should take place at the gut level. (34).

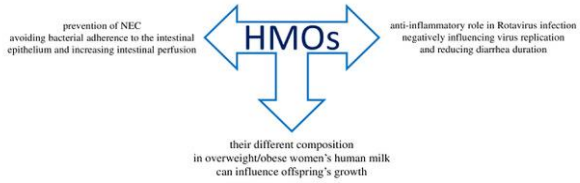

Figure 2. Role of human-milk oligosaccharides.

Also, HMOs have a role in neonatal rotavirus infection and disease presentation (35): although HMOs did not prevent the onset of rotavirus infection in animal models, it was demonstrated that they did reduce the duration of diarrhea by having an effect on colonic microbiota and the modulation of cytokine response induced by infection. No clear results are currently available in humans (36). An interesting recent study (35) has, finally, revealed that human oligosaccharides can influence the vaccine virus replication with either direct effects on virus replication or indirectly through modulation of the microbiome.

So, these biological factors can be used, with further studies, like a predictor of the oral Rotavirus vaccine response (37,38).

Finally, exclusive breastfeeding for at least two months could contribute to reduce intussusception in children under 5 years of age as recently observed in a large case control study conducted in Sicily (39).

4. Conclusions

It is clear that breastfeeding has many benefits such that WHO and UNICEF advise exclusive breastfeeding for 6 months (180 days) (40). However, many factors can influence the quality of human milk: the physical health of the mother, the breastfeeding method, mother's grade of atopy. Further studies will be necessary to evaluate the exact role of some components of human milk, such as exogenous antigens, ALP, IgA and how, vitamin supplementation can increase the well-known protective role of breastfeeding. Moreover, in the last few years, more and more importance has been given to HMOs and their pleiotropic effects (Figure 2). In the future, it will be necessary to outline the importance of the bioactive role of human milk and scientific progress, using metabolomic and proteomic research, could play a key role in achieving this.

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