Direct *vs.* indirect effects of bioactive milk proteins on neonatal growth: implications for failure to thrive and obesity.

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Abstract

The nutritive components of breast milk are well studied including the macronutrients, protein, lipid, and carbohydrates, as well as micronutrients such as vitamins and minerals. Recently, greater research attention has turned to other bioactive molecules found in milk that may play a significant non-nutritive role in normal infant development. Long chain-polyunsaturated fatty acids, complex oligosaccharides, bacteria, nucleotides, growth factors, and hormones are a few of these bioactive molecules found in breast milk. This mini-review examines research literature to determine if the growth and health promoting effects of bioactive proteins found in human breast milk are a result of intact, active molecules moving across the gut into infant circulation or the localized effect within the intestine. This topic has significant implications for both premature and failure to thrive infants as well as the opposite side of the spectrum with rapid, excess infant growth leading to childhood obesity.

Keywords: Breast milk, Infant formula, Cellular transport, Growth, Insulin-like growth factor, Leptin, Ghrelin, Adiponectin.

Introduction

Although, breast milk is touted as the superior nutritive fluid for complete infant nutrition, there are limited situations where formula is recommended in tandem or in replacement of breast-milk such as in cases of prematurity, failure to thrive, maternal drug use, or maternal HIV infection [1]. Development of infant formulas that closely align with the ideal breast milk formulation is an on-going research challenge. Breast milk contains optimal macronutrient (protein, lipid, and carbohydrate) content that is dynamic in response to infant needs; it is more easily digested than infant formula, and provides adequate minerals that are highly bioavailable [1]. Breast milk also provides immunological benefits to the infant resulting in fewer acute and chronic illnesses [1]. Breastfeeding is generally thought to be associated with reduced incidence of childhood obesity; however, this is still an active area of research with some new findings that may contradict these especially in cases of maternal obesity. The nutritive components of breast milk are well studied. Breast milk contains all essential amino acids primarily in the form of whey and casein proteins [1]. Lactose is the primary carbohydrate in breast milk while the lipid fraction of milk contains a variety of short, medium, and long chain fatty acids that are strongly influenced by maternal diet. Breast milk contains fat soluble vitamins (A, D, E and K), water soluble vitamins (C, riboflavin niacin, B6, B12, foliate and biotin) and minerals in adequate amounts to nourish an infant. Cellular components of breast milk include 0macrophages, neutrophils, T and B lymphocytes, and epithelial cells that provide immune benefits for infants. Innate immunity transfer from mother to infant is an important function of breast milk.

Recently, greater research attention has turned to other bioactive molecules found in milk that may play a significant non-nutritive role in normal infant development. Long chain polyunsaturated fatty acids, complex oligosaccharides, bacteria, nucleotides, growth factors, and hormones are a few of these bioactive molecules [2]. Proteins comprise the greatest diversity of these bioactive molecules. These bioactive proteins are intact functional enzymes, growth factors, hormones, and immune modulators that are found in physiologically relevant quantities in breast milk [2]. Table 1 provides a summary of important bioactive milk proteins. Research on the efficacy of these bioactive proteins in human milk is early in its progress. One significant question that has yet to be addressed is: are the growth and health promoting effects of bioactive proteins found in human breast milk a result of intact, active molecules moving across the gut into infant circulation or the localized effect within the intestine. This topic will be further examined within this brief review.

Table 1. Bioactive human milk proteins.

Protein	Bioactivity
Lactoferrin	Bacteriostatic; bactericidal; immunomodulatory; cell proliferation and differentiation
Alactalbumin	Prebiotic; antimictobial; immunostimulatory; enhanced Fe and Zn absorption
sigA	Transfer of maternal immunity; antibodies against bacteria and viruses
Lysozyme	Antibacterial activity; degredation of bacterial cell wall glycans
BSSL	Hydrolysis of triglycerides; fat absorption
Osteopontin	Immunomodulatory activity; brain function; intestinal development
Haptocorrin	Vitamin B12 absorption; antimicrobial activity
α1antitrypsin	Slows protein digestion
Bcasein	Opioid activity; enhancing calcium absorption
Kcasein	Antibacterial activity by acting as structural analogues
MFGM protein	Antibacterial and antiviral activities
*Leptin	Anorexigenic; regulation of adipose tissue; food intake; body weight
*Ghrelin	Orexigenic; promotes adiposity
*Adiponectin	Improves insulin sensitivity; increases fatty acid metabolism; reduces proinflammatory cytokines
*Insulinlike growth factorI	Growth factor; promotes cell proliferation and differentiation
*indicates protein hormones	

Implications/Importance

This topic has significant implications for both premature and failure to thrive infants as well as the opposite side of the spectrum with rapid, excess infant growth leading to childhood obesity. Studies have found a positive association with bioactive proteins and infant growth rate [3-6]. Preterm infant formula and breast milk is deficient in these bioactive proteins and may contribute to poor growth and health outcomes [2]. Human formula contains several bioactive hormones in greater quantities than breast milk including increased ghrelin and insulin-like growth factor (IGF)I and formula fed infants grow at a faster rate with increased adiposity compared with breast fed infants [7,8]. However, whether this is a direct effect on infant metabolism or an indirect effect of improved enterocyte development and function is unknown.

Issues with Obesity Macronutrients and Bioactive Proteins

Maternal obesity may contribute to childhood obesity due to genetic, epigenetic, or metabolic causes [9]. Maternal obesity results in the production of milk with greater protein content. Normal body mass mothers produce milk that is approximately 0.8% protein. Obese mothers' milk is between 1.0 to 1.2% proteins. The effects of macronutrient composition of breast milk on infant growth are well studied. Protein content of the milk is positively correlated with infant growth [5,10]. High protein content in milk increases circulating concentrations of insulin and IGF-I in the infant, and is associated with excess weight gain and later obesity [5]. In contrast, low protein formula reduced BMI and obesity risk [10]. Most research has investigated the exogenous protein intake resulting in increased endogenous IGF-I increase within the infant, while the contribution and effects of exogenous IGF-I and other metabolic hormones of the milk have been overlooked. This is in part due to the very difficult and complicated endogenous metabolic pathways that intertwine with the exogenous factors that modulate these pathways [9]. However, research has shown that obese mothers have greater insulin and leptin in their milk compared with normal weight mothers [4]. Further, these obese mothers have a greater incidence of obese infants compared with normal weight mothers.

In the past 5 years, several papers have examined the association of bioactive molecules in breast milk with infant growth rate and obesity [3-6]. Increased adiponectin and leptin in breast milk is associated with increased weight gain in infants [3,5]. Leptin in milk is positively correlated with maternal BMI [11]. This has been consistently found in all populations except ones experiencing significant environmental stress where no association was found [11,12]. An observational study monitored 103 mother infant pairs once per month for three months and measured IGF-I, leptin, ghrelin, adiponectin, protein and fat in breast milk [13]. This study found that independent of protein content, greater IGF-I and ghrelin in milk was associated with increased weight gain. These results further indicate the importance of bioactive protein hormones in normal and accelerated growth rate of human infants.

Further, these results support that hormones work in a manner independent of protein content of the milk. In contrast to the patterns described above with normal to overweight infants, preterm infant formula and breast milk is deficient in these

bioactive compounds and may contribute to poor growth and health outcomes [2]. Premature infants given early expressed breast milk did not result in increased plasma IGF-1; however, there was an association with early expressed milk and positive outcomes related to improved feeding which suggests improved enterocyte function [14]. These positive health outcomes lend support for increased IGF-I supplementation to preterm infants.

Bioactive Molecule Transport in Breast and Intestinal Tissues

Bioactive molecules that are found in breast milk can either be synthesized by the breast tissue or transported from blood capillaries through the basolateral membrane of the mammary alveoli and released at the apical surface of the alveolar epithelial cell into the milk collecting duct. Both processes are energetically expensive which would suggest that only molecules needed by the infant would be added to breast milk. Composition of metabolic proteins in milk changes across lactation [15]. Importantly, the concentrations of these metabolic proteins are often independent of maternal blood concentrations. Some of these proteins are found at much great concentrations in the milk compared with maternal blood suggesting active deposition in milk. McManaman et al. reviewed the transport mechanisms of molecules in breast tissue and briefly summarized below [16]. These mechanisms are similar to transport in other tissues with the exception of lipid molecules. Endogenously generated molecules in mammary epithelial cells are transported to the milk via exocytosis (pathway I) or lipid secretion (pathway II). Pathway I (exocytosis) is used for hydrophilic molecules such as milk proteins, oligosaccharides, lactose, citrate, phosphate, and calcium [16]. Hydrophobic milk lipids including phospholipids, triglycerides and lipid-associated proteins are transported via pathway II. This pathway involves a unique budding process where a membrane bound milk-fat globule is formed that includes some cytoplasm. For molecules produced elsewhere in the body three additional pathways exist. Transcytosis (pathway III) requires endocytosis at the basolateral surface, processing and sorting within the cell, and exocytosi at the apical surface of the breast epithelial cell. Pathway III is utilized by serum proteins, hormones, and immunoglobins. Membrane transport pathway (IV) is utilized by ions and small molecules such as glucose and amino acids. Pathway IV requires specific transport molecules at the both the basolateral and apical surface of the epithelial cell. The last pathway for molecules in breast tissue is paracellular (pathway V). This route is only accessible during pregnancy and involution due to the extensive tight junctions present in breast tissue during lactation [16].

Molecule movement in breast tissue is tightly regulated. Molecules that appear in breast milk have been synthesized by epithelial breast cells or actively transported from the blood plasma through the epithelial barrier into the mammary duct. Similar to mammary tissue, the epithelial cells within the intestine form a barrier for molecules to pass from the intestinal lumen into blood circulation. In young neonates the tight junction barrier may not be fully formed which may allow for unregulated passage of some molecules via the paracellular pathway. This early open gut is important for the passage of colostrum immunoglobins to infants for passive immunity. However, leaky gut can have negative health implications especially for pre-term infants. Improved gut closure and function could prevent pathological bacteria and other agents from penetrating the intestinal barrier as well as improving absorption of nutrients across the gut [17,18]. Preterm infants that received formula supplemented with IGF-I had a faster rate of gut closure than infants that did not receive IGF-I [19]. Lönnerdal et al. reviewed studies that examined the functions and activities of the proteins lactoferrin, α-lactalbumin, secretory IgA, Lysozyme, bile salt-stimulated lipase, osteopontin, haptocorrin, α-1-antitrypsin, β-casein, κ-casein and milk fat globule membrane proteins in breast milk, infant blood and feces. Of these 11 proteins many resist proteolytic degradation and therefore maintain active protein conformation and activity throughout the digestive tract [2]. This is an important finding. In order for these bioactive molecules to exert effects on enterocytes or within the body they must resist proteolytic degradation and remain functionally intact throughout the digestive tract. Similar studies examining metabolically active hormones in the digestive tract were not found in the literature search. However, research has made other discoveries that support the important role of these metabolic hormones in gut health and infant development.

Enteral nutrition is a critical part of gut development. Infants fed human breast milk have reduced GI-related disease and improved intestinal maturation compared with formula fed infants [18]. Bioactive proteins found in breast milk are key in this maturation and development. Alexander et al. found that administration of IGF-I to neonatal infants did improve gut intestinal barrier function; however they determined that the rate of closure was not improved enough to warrant this treatment recommendation [18]. IGF-I is important in gut development including both overall growth and function of the intestine [19,20]. IGF-I treatment increases protein content of the intestine, stimulates proliferation, suppresses apoptosis, increases rate of enterocyte maturation, and increases enzyme activity of the small intestine in new-borns [19]. Within the intestine leptin and IGF-I receptors have been found in the absorptive epithelial cells of the small intestine [19,20]. This suggests that bot molecules may be actively transported by the gut epithelial cell and transported into circulation. This hypothesis is further supported with studies that found a positive correlation between leptin concentration in breast milk and infant serum [20]. In piglets an increase in serum leptin was observed following consumption of colostrum with leptin in a dose dependent manner; however, growth effects of increased leptin consumption in neonatal pigs (<8 h old) did not result in increased growth at 24 days [21].

Significance and Future Research

Formula contains greater concentrations of IGF-I, ghrelin, and other metabolic hormones compared with breast milk. Infants fed breast milk have lower circulating IGF-I and reduced incidence of rapid growth leading to childhood obesity

compared with formula fed infants. Significantly, maternal obesity is the greatest predictor of childhood obesity. While research has focused on many non-physiologic factors that may contribute to childhood obesity, that fact that overweigh and obese mothers have greater insulin and leptin compared with normal BMI mothers, IGF-I promotes rapid enterocyte maturation and overall body growth, and rapid infant growth patterns are strongly predictive of childhood obesity suggests that Bioactive proteins including IGF-I and other metabolic hormones may play a key role in the physiology of obesity and physiological propagation of obesity between mother and child [4,9,17]. These bioactive proteins in breast milk and infant formulas should be a key focus in future childhood obesity research.

Understanding the role of bioactive proteins in normal infant growth is important for nutritional practitioners that may be investigating rapid infant growth leading to childhood obesity as well as impaired growth of premature infants. These bioactive proteins could be removed or limited in infant formulas to reduce growth rate, or compounds that bind and inactivate these proteins could be supplemented also to reduce growth rate. In the case of premature infant, IGF-I and other Bioactive proteins may be added to formulas improve intestinal function, intestinal maturation, and overall infant growth and health outcomes. The efficacy of these treatments is avenues of on-going and future research.

The original question investigated in the research was to examine if the growth and health promoting effects of bioactive proteins found in human breast milk a result of intact, active molecules moving across the gut into infant circulation or the localized effect within the intestine. Reasonable data support was found for the direct effects of bioactive proteins on enterocyte maturation, proliferation and function. Evidentiary support was also found to suggest bioactive protein hormones in milk increase circulating serum hormone concentrations within the infant leading to growth effects. However, the missing piece of evidence is confirmation that these Bioactive protein hormones in the gut are absorbed functionally intact through the intestinal barrier to result in a direct increase in circulating hormone concentration. Transport mechanisms do exist in mammary tissue for transcytosis (pathway III) of hormones; however, whether this pathway exists in the neonate intestine is unclear. An alternative route in early neonates would be the paracellular (pathway V); however, as the gut matures leaky membranes are quickly replaced with tight junctions. This pathway likely only remains available for a few days to weeks. Further research investigating the transport mechanisms for large, intact protein hormones in neonatal intestine need to be conducted to confirm if the increase in neonatal hormone concentrations are due to endogenous production or exogenous contribution from milk bioactive proteins.

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