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REVIEWS: CURRENT TOPICS

Longitudinal evolution of true protein, amino acids and bioactive proteins in breast milk: a developmental perspective☆

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Abstract

The protein content of breast milk provides a foundation for estimating protein requirements of infants. Because it serves as a guideline for regulatory agencies issuing regulations for infant formula composition, it is critical that information on the protein content of breast milk is reliable. We have therefore carried out a meta-analysis of the protein and amino acid contents of breast milk and how they evolve during lactation. As several bioactive proteins are not completely digested in the infant and therefore represent "non-utilizable" protein, we evaluated the quantity, mechanism of action and digestive fate of several major breast milk proteins. A better knowledge of the development of the protein contents of breast milk and to what extent protein utilization changes with age of the infant will help improve understanding of protein needs in infancy. It is also essential when designing the composition of infant formulas, particularly when the formula uses a "staging" approach in which the composition of the formula is modified in stages to reflect changes in breast milk and changing requirements as the infant ages.

Keywords: Human milk; Breast milk; Protein; Lactation; Infant nutrition; Amino acids

1. Introduction

Breast milk is an excellent source of protein and the preferred source of nutrition for infants. Breast-fed infants experience fewer and shorter infections [1,2], exhibit different growth patterns [2], have different gut microflora [3], show better cognitive development [4] and even face differences in the risk of chronic diseases, such as obesity [5,6], Type 1 and Type 2 diabetes [7–8] and cardiovascular disease [8,9]. Although the composition of infant formulas has evolved with increasing knowledge of infant nutrition, differences in outcomes between breast-fed and formula-fed infants still persist [10]. Efforts to improve outcomes of formula-fed infants and the composition of infant formula are complicated by variability in breast milk nutrient content. Human milk and its key components, including proteins, change continuously over time [11,12]. Consequently, narrowing the gap between breast milk and infant formula requires a greater understanding how protein quality and quantity in human milk changes over time.

Milk proteins are classified into three groups: milk fat globule membrane (MFGM) proteins, caseins and whey proteins. MFGM proteins contribute only a small percentage of the true protein content of human milk [13], a percentage that is likely relatively stable over time [14]. The principal proteins in human milk are caseins and whey proteins, which include α-lactalbumin, lactoferrin and secretory immunoglobulin A (sIgA). Concentrations of both casein and whey change profoundly over the course of lactation. Early in lactation, the concentration of whey proteins is very high, while casein is virtually undetectable [15,16]. As infants age, casein synthesis and, consequently, casein concentrations increase, partially due to hormonal changes in the mother. Because the amino acid contents of whey proteins and casein differ, milk amino acid content also changes as infants mature.

The protein intake of breast-fed infants has been used as a model for infant protein requirements, given that breast milk is typically the only source of protein before complementary foods are introduced. Protein content in breast milk can be quantified by directly assessing the true protein content or quantifying the nitrogen content in breast milk. True protein can be calculated from the nitrogen content by subtracting nonprotein nitrogen from the total nitrogen and multiplying the difference by a conversion (Kjeldahl) factor [14].

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Assessments of true protein content using these methods have reported concentrations of 14 to 16 g/l during early lactation, 8 to 10 g/l at 3 to 4 months and 7 to 8 g/l at 6 months [14,16,17]. However, true protein intake does not accurately reflect the amount of utilizable amino acids in infants because some breast milk proteins can be found intact in infant stool [18]. Lactoferrin and sIgA, for example, are found in relatively high amounts in feces from breast-fed infants.

These proteins — and many others — have important roles in breast milk beyond nutritional support. Bioactive proteins likely contribute to the numerous advantages of breast milk over infant formula. Bioactive proteins can have enzymatic activity, enhance nutrient absorption [19], stimulate growth [20], modulate the immune system [21] and assist in the defense against pathogens [21–24]. Key bioactive proteins in human milk include lysozyme, α-lactalbumin, κ-casein and β-casein, as well as lactoferrin and immunoglobulins, especially sIgA (Table 1) [10,14,24].

The extent to which true protein, amino acid and bioactive protein content in breast milk changes over time has been evaluated in a variety of studies using different methodologies. A single reference that analyzes true protein content, amino acid content and bioactive protein content, describes their changes throughout the course of lactation and considers the implications of these changes in infant development is needed and would be useful to efforts to improve infant nutrition.

To meet this need, we conducted a meta-analysis and literature review to evaluate changes in these protein parameters during infants' first year of life. In this meta-analysis and review, we compiled and analyzed data on protein and amino acid content in human milk available in the medical literature. We used this dataset to estimate the longitudinal evolution of total protein, amino acid and certain bioactive protein content in human milk from birth through 1 year. We also interpreted these changes in the context of the known and proposed biological functions of the evaluated proteins. It is hoped that this analysis will provide a reference dataset for changes in protein content over time, a dataset that can be used to improve our understanding of the protein and amino acid intake of breast-fed infants and to enhance the composition, staging and performance of infant formulas.

2. Methods

2.1. Literature search

To identify all published literature on protein content of breast milk, we performed literature searches using PubMed, Scopus, EMBASE and Google Scholar using the following keywords: *breast milk*, *human milk*, *protein*, *true protein*, *total protein nitrogen*, *protein nitrogen*, *bioactive proteins*, *whey to casein ratio*, *lactoferrin*, *α-lactalbumin*, *serum albumin*, *IgA*, *lysozyme*, *IgG*, *IgM* and *amino acid*. The most recent search was conducted in March 2015. Reference lists of the retrieved articles were also reviewed to identify references not found using electronic search methods. Only data from "normal" or "healthy" mothers who delivered healthy term infants were included in this meta-analysis. Studies evaluated mothers who consumed free-living diets; data from mothers consuming special diets were excluded. Selected studies provided sufficient information regarding geographic location, study design, sampling time and procedure, nature of sample, analytical methods and units. Other variables such as age, ethnicity, body weight, socioeconomic status and season were not considered. Milk could be obtained with mechanical, electrical and hand pumps or by manual expression. Samples were transported and stored in either liquid or freeze-dried form; defatted or whole milk was used for hydrolysis. Milk samples analyzed were taken from complete 24-h collections, the entire amount of milk from one or both breasts at one feeding or pooled or banked milk.

2.2. Data extraction

Data were extracted from studies that reported true protein content, protein-bound amino acid content and bioactive proteins. Assessments of bioactive proteins included evaluations of whey-to-casein ratios and concentrations of lactoferrin, α-lactalbumin, serum albumin, sIgA, lysozyme, immunoglobulin G (IgG) or immunoglobulin M (IgM). Total, essential and nonessential amino acid content was evaluated in available studies. *Protein quality* was defined as the ratio of essential to nonessential amino acid concentrations. For protein content analysis, total nitrogen data were not considered relevant, and only true protein data obtained using Kjeldahl (total nitrogen — nonprotein nitrogen with a 6.25 conversion factor), Lowry, Biuret and bicinchoninic acid (BCA) kits were extracted. When other conversion factors (*i.e.*, 6.38) were used to estimate true protein content using the Kjeldahl method, the data were recalculated using 6.25 as conversion factor. Data summaries were prepared using the means from original reports converted into consistent units (g per 100 ml, mg per 100 ml or mg per ml). When sampling time was provided as ranges and not specific days, sampling time was calculated based on the average lactation day. Data were categorized by stage of lactation as follows: colostrum (0 to 5 days postpartum), 6 to 15, 16 to 30, 31 to 60, 61 to 90 and 91 to 360 days postpartum. Means, medians, 25th and 75th percentiles, minima, maxima and standard deviations were calculated and used to prepare summary tables and graphical plots. Linear regression of the true protein dataset was performed using R Version 3.0.1.

3. Results

Separate analyses were conducted for each evaluated endpoint. A total of 43 original articles published between 1953 and 2011 were included in at least one analysis.

3.1. True protein

In our evaluation of true protein content, we considered 34 original articles published between 1973 and 2011. Eight of these papers were excluded due to unreliable analytical methodologies, leaving 26

Table 1
Included studies on true protein content in human milk

First author	Year	Country	Study design
Allen [101]	1991	USA	Longitudinal
Andersson [102]	1983	USA	Longitudinal
Arnold [103]	1987	Australia	Longitudinal
Bauer [104]	2011	Germany	Longitudinal
Britton [105]	1986	USA	Longitudinal
Butte [106]	1984	USA	Longitudinal
Butte [107]	1984	USA	Longitudinal
Butte [108]	1990	USA	Cross-sectional
Dewey [109]	1983	USA	Longitudinal
Gross [110]	1980	USA	Longitudinal
Harzer [111]	1986	Germany	Longitudinal
Hibberd [112]	1982	UK and Germany	Longitudinal
Kunz [16]	1992	USA	Longitudinal
Lönnerdal [26]	1976	Sweden	Longitudinal
Marquis [32]	2003	Peru	Longitudinal
Mitoulas [113]	2002	Australia	Longitudinal
Montagne [114]	1999	France	Longitudinal
Nagasawa [115]	1973	Japan	Cross-sectional
Nagra [34]	1989	Pakistan	Longitudinal
Nommsen [116]	1991	USA	Cross-sectional
Ronayne de Ferrer [28]	2000	Argentina	Longitudinal
Saarela [11]	2005	Finland	Longitudinal
Sanchez-Pozo [27]	1986	Spain	Longitudinal
Sann [117]	1981	France	Longitudinal
Shehadeh [118]	2006	Israel	Cross-sectional
Stuff [119]	1989	USA	Longitudinal