No. 42 / April 2018

the nest

Human Milk: Evolving of Nature's Understanding

Nestlé Nutrition Institute

Peptides in Human Milk

J. Bruce German

Foods for Health Institute
University of California, Davis, CA, USA
jbgerman@ucdavis.edu

Key Messages

Milk proteins undergo digestion into peptides and amino acids within the stomach and intestine in a complex series of reactions.

Many of the peptides from milk are generated by endogenous enzymes within the mammary gland prior to secretion.

Peptides from milk proteins in turn are generated by milk's endogenous enzymes throughout the gastrointestinal tract of the infant.

The presence of peptides in human milk is well known, and research on these components has been active for over half a century. The history of peptide research and innovation can be thought of as occurring in 3 phases. During the first period of scientific discovery and applications, milk proteins were digested by microbial proteases into peptides prior to consumption with the goal to enhance digestion and minimize immunological reactions of the intact proteins. The second phase focused on peptides released by specific proteases with explicit biological sequence and activities screened for known targets of therapeutic benefit. The third phase (just beginning) is focusing on the peptides actually produced within the intestine of breast-fed infants, and research is identifying the targets of their action within infants.

Milk proteins undergo digestion into peptides and amino acids within the stomach and intestine in a complex series of reactions guided by proteolytic enzymes, physical mixing, pH shifts, and biological surfactants. Not surprisingly given the complexity, digestion can fail to reach completion both due to insufficient capacity within individuals due to immature digestive system or health conditions and to unusually resistant protein structures in the diet. If undigested, proteins and large peptides continue to flow down the intestine, fail to deliver amino acids as nourishment, can elicit autoimmune reactions notably allergies, and feed specific members of the intestinal microbial community yielding undesirable products and metabolites. In response to the documented consequences of failures in protein digestion, scientists developed strategies to pre-digest proteins and provide partially hydrolyzed products [1].

Some peptides released from milk have bioactive properties. Anti-microbial and antihypertensive screens were the first to yield positive results [2].

The arrival of genomics and the modern analytical methods has made it possible to identify, sequence, and annotate the protein origins of the peptides in human milk that are actally generated in vivo within the mammary gland and infant [3]. This new approach has the potential to change the entire perspective of peptide efficacy. Many of the peptides from milk are generated by endogenous enzymes within the mammary gland prior to secretion [4]. Peptides from milk proteins in turn are generated by milk's endogenous enzymes throughout the gastrointestinal tract of the infant.

While it is not guaranteed that all peptides generated in milk evolved to provide specific biological functions, it is a compelling place to start. Their actions in human infants act as food, i.e. consumed biomaterials, reach their targets of efficacy at an effective dose, and, perhaps most importantly, do so safely. As the targets of these peptides are discovered, it will be possible to develop diagnostics to monitor those processes, new strategies, and new products. A bright future indeed.

References

1. Greer FR, Sicherer SH, Burks AW; American Academy of Pediatrics Committee on Nutrition; American Academy of Pediatrics Section on Allergy and Immunology: Effects of early nutritional interventions on the development of atopic disease in infants and children: the role of maternal dietary restriction, breastfeeding, timing of introduction of complementary foods, and hydrolyzed formulas. Pediatrics 2008;121:183–191. doi: 10.1542/peds.2007-3022. Review.
2. Clare DA, Swaisgood HE: Bioactive milk peptides: a prospectus. J Dairy Sci 2000;83:1187–1195. doi: 10.3168/jds.S0022-0302(00)74983-6.
3. Dallas DC, Guerrero A, Khaldi N, Borghese R, Bhandari A, Underwood MA, Lebrilla CB, German JB, Barile D: A peptidomic analysis of human milk digestion in the infant stomach reveals protein-specific degradation patterns. J Nutr 2014;144:815–820.
4. Guerrero A, Dallas DC, Contreras S, Chee S, Parker EA, Sun X, Dimapasoc LM, Barile D, German JB, Lebrilla CB: Mechanistic peptidomics: factors that dictate the specificity on the formation of endogenous peptides in human milk. Mol Cell Proteomics 214;13:3343–3351.

Temporal Evolution of Human Milk Oligosaccharides

Sean Austin[1]
Norbert Sprenger[2]

[1] Institute of Food Safety & Analytical Science, Nestlé Research Center, Nestec. Ltd., Lausanne, Switzerland
sean.austin@rdls.nestle.com
[2] Institute of Nutritional Science, Nestlé Research Center, Nestec. Ltd., Lausanne, Switzerland
norbert.sprenger@rdls.nestle.com

Key Messages

Human milk oligosaccharides are a major component of human milk

Over 200 human milk oligosaccharides (HMO) are predicted to be present in human milk (1)

The HMO profile differs between individuals and changes during the course of lactation

While globally HMO levels decrease during lactation, individual HMO have differing rates of change, and for some the concentration increases during lactation.

HMO are the third most abundant solid component of human milk after lactose and lipids and are generally not digested by the newborn. They are postulated to protect the infant from infections by (i) preventing the binding of pathogenic bacteria to host cells, (ii) helping in the establishment of the gastrointestinal microbiota, and (iii) modulating the developing immune system [1]. Some may act as a conditional dietary source of sialic acid [1].

The levels and types of HMO in breastmilk differ among individuals primarily depending on their expression of various enzymes called glycosyltransferases. The glycosyl transferases are responsible for elongating lactose with residues of N-acetylglucosamine, galactose, fucose, and/or sialic acid, and as such, the production of different HMO.

The total HMO content in milk decreases during lactation, presumably reflecting the changing needs of the developing infant. HMO content in colostrum may be over 20 g/L, decreasing to around 13 g/L at 4–6 months of lactation [2]. Recent research suggests that the HMO content may slightly increase again during the second year of lactation [3]. The behaviour of individual HMO are not the same (Fig. 1). For example, while the concentration of 2′-fucosyllactose (2′FL) decreases over the first few months of lactation, the concentration of 3FL increases. Also those that decrease do not all decrease at the same rate. 6′-sialyllactose (6′SL) is generally regarded as the predominant SL in human milk. While this is true at early stages of lactation, the concentration of 6′SL reduces much more dramatically than that of 3′SL, leading to an increased proportion of 3′SL at later stages of lactation.

The differing rates of syntheses of individual HMO suggests that they most likely play different biological roles, adapting to the growing infant's physiological needs [4]. As our knowledge of the dynamics and functions of the different HMO during infant development increases, we will better understand the changing needs for these in the growing infant's diet.

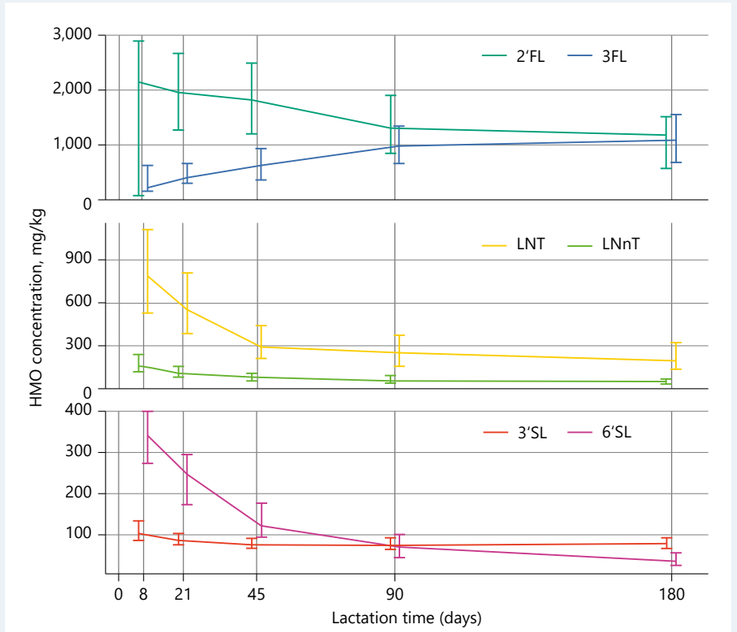

Fig. 1. Median levels with interquartile ranges over time of lactation of key individual HMO, representative for the fucosylated (2′FL, 3FL), neutral non-fucosylated (LNT, LNnT), and sialylated (3′SL, 6′SL) HMO [5]. 2′FL, 2′-fucosyllactose; 3FL, 3-fucosyllactose; LNT, lacto-N-tetraose; LNnT, lacto-N-neotetraose; 3′SL, 3′-sialyllactose; 6′SL, 6′-sialyllactose

References

1. Oliveira DL, Wilbey RA, Grandison AS, Roseiro LB: Milk oligosaccharides: a review. Int J Dairy Technol 2015;68:305–321. http://dx.doi.org/10.1111/1471-0307.12209.
2. Coppa GV, Gabrielli O, Pierani P, Catassi C, Carlucci A, Giorgi PI: Changes in carbohydrate composition in human milk over 4 months of lactation. Pediatrics 1993;91:637–641.
3. Perrin MT, Fogleman AD, Newburg DS, Allen JC: A longitudinal study of human milk composition in the second year postpartum: implications for human milk banking. Matern Child Nutr 2017;13. http://dx.doi.org/10.1111/mcn.12239.
4. German JB, Freeman SL, Lebrilla CB, Mills DA: Human milk oligosaccharides: evolution, structures and bioselectivity as substrates for intestinal bacteria. Nestle Nutr Workshop Ser Pediatr Program 2008;62:205–218; discussion 218–222. http://dx.doi.org/10.1159/000146322.
5. Austin S, De Castro CA, Benet T, Hou Y, Sun H, Thakkar SK, Vinyes-Pares G, Zhang Y, Wang P: Temporal Change of the Content of 10 Oligosaccharides in the Milk of Chinese Urban Mothers. Nutrients 2016; 8: 346. http://dx.doi.org/10.3390/nu8060346.

Protease Enzymes in Human Milk

J. Bruce German

Foods for Health Institute
University of California, Davis, CA, USA
jbgerman@ucdavis.edu

Key Messages

Human milk evolved not as simple proteins but rather as a combination of proteins and protease enzymes.

Young infants are developmentally naïve, produce little gastric acid, and express low protease activity.

The selective proteolysis of milk proteins begins within the mammary gland.

Introduction

The emergence of lactation as the system of nourishing infants has been a core asset to the success of mammalia. The traits of lactation selected within the mother infant dyad has been a Darwinian engine of nourishment for over 200 million years [1]. The proteins of milk are proving to be an even more complex nutrition and protection system than previously considered. Scientists are now using modern tools of biological research to understand milk proteins and their digestion into peptides within infants [2, 3]. Mapping these peptides to the proteins that contained them and the sites that were cleaved revealed a surprising result: the protease enzymes defined by their cleavage specificity correspond to enzymes not in babies but in the milk [4]. A total of five protease enzymes (plasmin, cathepsin, elatase, kalikrein, and amino- and carboxypeptidase) were identified to be either translocated to or synthesized in the mammary gland, present in milk, and active within the infant stomach. These results suggest that milk evolved not as simple proteins but rather as a combination of proteins and protease enzymes.

Implications

First: the infant. The current paradigm for protein nourishment is that intact proteins denatured by stomach acid and attacked by endogenous proteases in the stomach begin a digestive process that continues with hydrolysis by neutral proteases in the small intestine, and ultimately leads to the release and complete absorption of amino acids by the intestinal epithelia. Young infants, however, are developmentally naïve, produce little gastric acid, and express low protease activity. Nonetheless, infants digest and absorb milk proteins effectively with an array of proteases activated within the infant and contributing to catalytic activity. Specificity to protein digestion within the infant has some important implications to infant nourishment.

Second: the milk. There are no means at hand to measure – and much less to deliver – this aspect of human milk to all infants. Sharing of breast milk, storing, processing, all will affect the net ability of milk's enzymes to selectively digest the proteins. Formula does not currently contain these activities. Scientific discovery must now identify all peptides released, where and when, annotate their functions, and understand their value to infants.

Third: the mother. The selective proteolysis of milk proteins begins within the mammary gland. Are these activities of benefit to the mother, to lactation? How diverse are these activities across mothers, across lactation, across varying maternal health and nutritional status? Answers to these questions will guide future policies and practices of lactation.

Understanding this magnificent system of nourishment is likely to provide insights for nourishing humans of all ages and all health conditions.

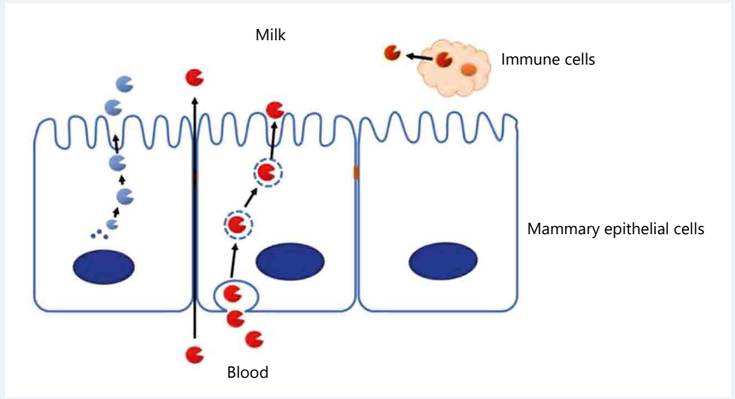

Fig. 1. Schematic of production and transfer of enzymes into milk within the lactating mammary gland. Enzymes can reach milk by direct protein synthesis by the epithelial cell, by translocation from blood, and by secretion from immune cells within the mammary gland and milk itself

References

1. Oftedal OT: The mammary gland and its origin during synapsid evolution. J Mammary Gland Biol Neoplasia 2002;7:225–252.
2. Dallas DC, Guerrero A, Khaldi N, Castillo PA, Martin WF, Smilowitz JT, Bevins CL, Barile D, German JB, Lebrilla CB: Extensive in vivo human milk peptidomics reveals specific proteolysis yielding protective antimicrobial peptides. J Proteome Res 2013;12:2295–2304.
3. Dallas DC, Guerrero A, Khaldi N, Borghese R, Bhandari A, Underwood MA, Lebrilla CB, German JB, Barile D: A peptidomic analysis of human milk digestion in the infant stomach reveals protein-specific degradation patterns. J Nutr 2014;144:815–820.
4. Khaldi N, Vijayakumar V, Dallas DC, Guerrero A, Wickramasinghe S, Smilowitz JT, Medrano JF, Lebrilla CB, Shields DC, German JB: Predicting the important enzyme players in human breast milk digestion. J Agric Food Chem 2014;62:7225–7232.

The material contained in this booklet was submitted as previously unpublished material, except in the instances in which credit has been given to the source from which some of the illustrative material was derived.

Source of illustrations:
Nestlé Nutrition Collection

Great care has been taken to maintain the accuracy of the information contained in this booklet. However, neither Nestlé Nutrition Institute nor S. Karger AG can be held responsible for errors or for any consequences arising from the use of the information contained herein.

Published by S. Karger AG, Switzerland
for Nestlé Nutrition Institute.
Wyeth Nutrition Science Center is
part of Nestlé Nutrition Institute
CH–1800 Vevey
Switzerland

ISSN 1270–9743

To learn more about the Nestlé Nutrition Institute
and Wyeth Nutrition Science Center resources, visit:
www.nestlenutrition-institute.org
www.wyethnutritionsc.org

Nestlé Nutrition Institute