Milk: A Model of Diet and Health for the 21st Century

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Take Home Messages

- Human health is complex, dynamic and personal in which each individual's genetics, lifestyle and diet interact to influence their health trajectory and outcome
- The future of health management for disease prevention will require a more personal approach in practice combining direct measures of health status with diet and lifestyle guidance
- To achieve prevention, the health sciences need to establish what to measure within individuals to provide actionable information about their current health status and the targets on which dietary components act to promote and protect health within healthy individuals
- Milk and lactation evolved under the constant Darwinian selective pressure to be nourishing, protective and supportive of the health of healthy mammalian infants
- The lessons from 200 million years of evolution within these constraints provide molecules, mechanisms and benefits to guide research and application of diet for the 21st century
- The emergence of indigestible oligosaccharides as a component in milk that selects and nourishes a protective microbiota is a vivid example of the insights into diet and health that milk provides

Introduction

The sciences related to diet and health have succeeded in identifying all of the essential nutrients and the diseases associated with their deficiency. The goal is now to build an integrative view of diet and health including a more personal view of the variations in health of individuals and a comprehensive view of entire diets and lifestyles as system ensembles. To this end, it will be

WCDS Advances in Dairy Technology (2011) Volume 23: 5-16

necessary to assess human health and acquire models of successful diets that can guide scientific research toward new foods.

The science of genomics has established complete sequences of humans and dozens of plants and animals and literally hundreds of microorganisms, yeast, bacteria and viruses. These genomes of different organisms are being annotated as a concerted scientific push to understand biology and biological processes in genetic detail. This understanding can extend to diet and health by interrogating genomes and evolution from the perspective of nourishment. As the product of millennia of Darwinian selective pressure as a complete diet, mammalian milks are proving to be a gold mine of biological knowledge precisely to understand nourishment.

■ Diet and Health

The 21st century will be the century of biology. All of the Life Sciences will be guided by the developing biological understanding of the genetic basis of life following on the sequencing of the entire genome of humans, of hundreds of viruses, of bacteria, plants and animals, and even entire ecosystems. Scientists are examining human processes with the tools of genomics. Nutrition has already launched the field of Nutrigenomics and begun its commercialization into personalized diets. Genomics has not been as active in examining the genetics of food materials for their evolutionary functions. Now, lactation and milk are being addressed (Lemay et al., 2009, Elsink et al., 2009). These studies have not yet led to a reconsideration of how diet itself should be studied. In pursuit of novel bioactivities from foods, researchers have explored the responses of experimental animals and even humans to various dietary ingredients from plants. The assumption is that plants are most nourishing. This is not unexpected given the fact that plants are responsible for the production of the essential vitamins, amino acids and fatty acids and are typically adequate sources of essential minerals. However, with the now mature knowledge of the essential nutrients, the value of plants to provide useful information beyond these is not as apparent. In fact, from the perspective of evolution, plant genomes will provide insights into how to avoid being consumed by animals, not how to nourish, protect and promote their health. To be able to profit immediately from genome research, future research should focus on milk and lactation for guiding principles.

■ The Evolution of Milk

Research on the basic production of milk as an agricultural commodity has led to a substantial knowledge of mammary epithelial cell biology through the various stages of lactation, the physiology of the mammary gland, the

physiology and supporting role of non-epithelial tissues, the energetics of lactation, the regulation of genes and proteins expressed during lactation, the nourishment of the lactating gland and the biochemical processes that occur within lactating mammary epithelial cells and the mammary gland (German et al., 2006).

The genomes of several mammals and the bovine genome sequencing project has been completed (Elsink et al., 2009; Lemay et al., 2009). A remarkable investment across the entire mammalian genome has been made in lactation, both as direct gene products, i.e. proteins and as indirect products of lactation-specific enyzmes, lipids, carbohydrates, etc. (Lemay et al., 2009). The genes of lactation encode the products, functions and regulation of milk component synthesis and secretion by the mammary gland. The genes of lactation also dictate the regulation of the mammary gland; its structural and physiological remodeling, its metabolic capacity and its protection including the diversity of the various reproductive strategies of different mammals. To understand a mammal's genome related to lactation it is necessary to understand what milk is - a secretion made by the differentiated mammary gland. This essential food of all mammalian life is principally composed of water, lipids, protein (largely casein and whey), and the carbohydrate lactose and other oligosaccharides. Nonetheless, across mammals, there is a wide diversity of lactation strategies according to the biology of the mother, infant and ecological demands during infant feeding. The variations in milk composition and functions across species presumably reflect the need to adapt to the environmental niche, reproductive strategy, and nutrient and growth requirements of the neonate.

Research to determine the biological functions of milk components once consumed is challenging. Milk components are disassembled within the stomach and intestine, absorbed and transported to various tissues and their discrete actions are difficult to assign to mechanisms. The overall success of the young mammalian infant in a hostile world attest to milk's success. How to assign components to discrete functions remains elusive. Genomics provides a new path to that discovery process.

■ Discovering Milk's Functions

The milk genome is defined in this article as all genes responsible for lactation (Ward and German 2004; Lemay et al., 2009). This simple idea is somewhat naïve considering the complexity and dynamics of lactation, nonetheless serves as a conceptual framework for discussion. The genes associated with lactation extend beyond the mammary tissue and the period of lactation. Lactation requires processes outside of the mammary gland to support the development of the gland as a bioreactor. Many of these events occur before lactation. Similarly once lactation ceases, extensive remodeling of the gland

must occur to return it to the pre-lactation state. Using conservative statistical multiple testing corrections ($p<0.001$), nearly a third of the mouse transcriptome is in flux from pregnancy through involution (Lemay et al., 2007). There is no sudden transcriptional switch around the time of parturition but instead, pregnancy is a period of a broad range of progressive developments in which the timing of onset of lactation is established. Interestingly, the cessation of lactation does appear to have a switch, at least a substantial proportion of the lactation genes are switched off in a coordinated fashion. How this switch is fully accomplished is a subject of intense research. Controlling lactation and the variation of milk composition would be an immediate application of understanding the onset of lactation processes during pregnancy and the onset of involution switching mechanisms.

■ The Health Benefits of Milk

The emergence of the mammary gland in evolution occurred well over 200 million years ago (Lefebvre et al., 2010). Once 'discovered' the descendents of this remarkable feeding invention have enjoyed remarkable success and mammals today largely dominate the planet. Through the gradual evolution of milk's components, the steps presumably were the adaptive recruitment of existing precursor genes through alteration of regulatory sequences to allow expression in primitive mammary glands and, duplication and mutation of structural sequences to acquire new functions from pre-existing primitive proteins. The earliest mammary function perhaps even prior to the provision of nutrition was the passing of protective advantages on to offspring by immunoglobulins, thus aiding selection for survival. Milk can thus be seen as a biological strategy rich in protective functions, nourishment of infant offspring; disease defense for the infant; disease defense for the mother; regulation or stimulation of infant development, growth or function; regulation or stimulation of maternal mammary tissue development, growth or function; inoculation, colonization, nourishment, regulation and elimination of infant microflora; and inoculation, colonization, nourishment, regulation and elimination of maternal mammary microflora. The scientific understanding of these functions will provide the means to control them and their value to the entire population.

■ Milk and the Infant

Nourishing the mammalian neonate is considered job one for milk. All of the essential macronutrients, water, vitamins, minerals, amino acids and fatty acids, plus the structural and energetic intermediates needed to sustain life, must be delivered to the neonate in an absorbable form appropriate to the

species and the stage of development. What is not as recognized is the cost to the mother. All of these components, including the energy, cost the mother. Lactation research has illuminated many of the processes needed to mobilize the essential biomolecules from maternal stores. To date, the molecular strategies of converting them into dispersed, transportable and bioavailable structures in milk have been more difficult to translate into other foods.

Milk is not just a source of the essential nutrients. The non-essential components of milk and their roles are being pursued (German et al., 2002). Nonetheless, studies needed to discover these properties are different from those used to discover the properties and roles of essential nutrients. Essential nutrients can be studied with relative ease because their elimination from the diet of animals leads to overt deficiency in every individual. Non-essential nutrients and their functions, however, are valuable only in context, and research has not been as successful in defining the contexts in which non-essential nutrients are valuable.

■ Beyond Essential Nutrients

Milk provides benefits to infants that improve their probability of survival. To date, research into proteins and their biological activities has demonstrated values to health mechanisms in vitro, in animal models and in infant and adult humans (Lönnerdal, 2003, 2004). Still we cannot explain why infants fed not on human milk but on formulas with all essential nutrients experience different growth patterns (Dewey, 1998), different nutritional status (Castillo et al., 1996), more infections of longer duration (Oddy, 2001) and different gut microflora compared to infants fed breast milk (Rubaltelli et al., 1998). Physiological benefits provided by milk in the gastrointestinal tract include enhancement of nutrient absorption, enzyme activities, growth stimulation, modulation of the immune system and defense against pathogens (German et al., 2002).

One of milk's well described properties is the protection against infections including: immunological factors (antibodies, cells, cytokines), proteins (lactoferrin, enzymes, e.g., lysozyme), oligosaccharides and glycoproteins, gut microflora (prebiotics) and nutrients to optimize the infant's immune system. Antimicrobial activity is provided by iron-dependent bacteriostatic and iron-independent bactericidal factors; antiviral factors inhibit viral binding to cells and inhibit virus infection; immunoregulatory factors modulate lymphocyte and monocyte responses, binding factors, immune maturation factors and overt microbial binding factors (Stelwagen et al., 2008; Politis and Chronopoulou, 2008; Admyre et al., 2007). Antimicrobial proteins, such as lactoferrin, lysozyme and haptocorrin, a vitamin B_{12}-binding protein in human milk (Adkins & Lonnerdal, 2003), may exert a host defense function against pathogens in the gastrointestinal tracts of breastfed infants.

Beyond protection from pathogens, milk appears to contain factors that assist with intestinal and circulatory functions: opioid agonists that inhibit gut motility and reduce digesta passage rate; opioid antagonists that block opioid effects on gut; antihypertensive factors that inhibit angiotensin-converting enzyme, reduce vasoconstriction and increase blood flow; antithrombotic agents that inhibit platelet activation and reduce clot formation; cell regulation factors that modulate cell proliferation and induce apoptosis (Rutherfurd and Gill, 2000; Wagner et al., 2008).

Difficulties in assigning the bioactivities of milk are in part because they are latent, i.e., they are inactive while "encrypted" within the intact milk protein (Fitzgerald et al., 2004) and only become active once released from the parent milk protein by proteolytic activity. Proteins within milk that produce bioactive peptides when hydrolyzed are β-casein, α_{S1}-casein (α-casomorphin, an ACE inhibitor); κ-casein, about 40% glycosylated (glycomacropeptide, an antithrombotic peptide formed during proteolysis); β-lactoglobulin (β-lactorphin); α-lactalbumin (α-lactorphin); lactoferrin (lactoferroxins—bactericidal peptides, including an immunoregulatory peptide and an antibacterial peptide) (Meisel and Fitzgerald 2003; Rutherfurd and Gill 2000).

■ Self-assembling Structures in Milk

Milk is an obligate liquid, exiting the mammary epithelia through very small pores, hence it is not considered to be a highly structured food. Nonetheless, milk provides many structures both those that are present at secretion and others that form within the infant's intestine. Unfortunately, to date, little research has addressed the biological values of these structures either to infants or to adults (Ward et al., 2004). Milk is nonetheless a model for foods with self-assembling components. As scientists begin to understand the more structure-specific properties of molecules, then the self-assembly properties of biomolecules, even complex colloidal properties, could be understood and controlled (de Campo et al., 2004). If the principles of structure formation in milk could be understood, the same biophysical principles could be applied to formulate, stabilize and texturize a variety of other food products, without the macroscopic, high-energy unit operations currently used today (Leser et al., 2006). Cheese illustrates this principle. Casein micelles aggregate into three-dimensional gel networks that provide unique textural properties to cheese. Proteins are not the only biomolecules in milk that form complexes however and the higher order structures found in milk and those that spontaneously assemble while it is being digested are targets of ongoing research (Argov et al., 2008).

■ Microbial Responses to Milk

Milk Oligosaccharides

Milk is the output of a dynamic interplay between the resources of the mother and the needs of the infant. Everything in milk costs the mother. If it does not improve the health of the infant, its cost to the mother would put it at a significant selective disadvantage. However, anything in milk that improves the health of an infant and increases its success puts that component under positive selective advantage. Given this maternal – infant conflict, it is astonishing to recognize that human milk contains abundant free oligosaccharides that are non-digestible by the mother nor infant. Since they cannot provide nourishment directly, investigators have suggested physiological and protective functions to these molecules (Coppa et al., 1993, Kunz et al., 2000; Newburg 2005; Bode, 2006). Yet, the evolutionary pressure that led to the structural diversity of these molecules is not yet understood. Human milk oligosaccharides have been demonstrated to selectively nourish the growth of highly specific strains of bifidobacteria thus guiding the development of a unique gut microbiota in breast milk fed infants (Ward et al., 2006; Ninonuevo et al., 2007).

Human milk contains a mixture of oligosaccharides composed of both neutral and anionic species with building blocks of 5 monosaccharides: D-glucose, D-galactose, N-acetylglucosamine, L-fucose, and N-acetylneuraminic acid (Ninonuevo et al., 2005). The core structure of HMO is lactose. The lactose core is elongated by N-acetyllactosamine units, with extensive fucosylation and/or sialylation (Newburg, 2005). Over 100 molecular species have been identified in a pooled human milk sample consisting of mostly neutral and fucosylated oligosaccharides (Ninonueva et al., 2006).

The biochemistry behind the synthesis of these molecules in the mammary gland and its regulation remains poorly understood as the variations between mothers has only recently been documented (Ninonueva et al., 2008). It is not clear if the variation in humans is due to genetic polymorphism, metabolic differences or nourishment of the mother. It is also not known if these differences between infants in different breast feeding situations translate into differences in microbiota and health outcomes.

Microbial fermentation of Milk Oligosaccharides

Oligosaccharides orchestrate the intestinal ecosystem (microbiota) by selectively stimulating the growth of only a few bacterial (Ward et al., 2006; Locascio et al., 2008). The entire sequence of one of these organisms has now been completed and its genome is proving to be an example of co-evolution of bacteria and humans (Sela et al., 2008). Consistent with such an

ecological adaptation, a significant portion of the genome of B. infantis is apparently dedicated to genes responsible for digesting, transporting and metabolizing the oligosaccharides of human milk. The genes encoding the enzymatic capabilities are not randomly distributed across the bacterial genome, but instead are localized to 4 distinct clusters. In fact most of the genes associated with degradation of the oligosaccharides from milk are located in a single genomic cluster.

These bacteria are present and successful within their ecological niche, the human infant intestine. Their success in outcompeting other bacteria is at least in large part the availability of the undigestible unabsorbable oligosaccharides reaching the lower intestine in a breast fed infant. The simple presence of these bacteria does not explain the benefits that these bacteria provide the infant within this symbiotic relationship.

■ Conclusions

There is a pressing need for scientific research in the 21st century to build the detailed molecular, mechanistic and systemic knowledge of human health and disease. The agricultural enterprise must deliver safe, convenient, affordable, desirable and highly nourishing food products that provide consumers the means to assemble diets that maintain and improve their health and prevent disease. Nutrition research has already demonstrated its power to solve diet-related health problems by identifying the essential nutrients, recognizing their absolute requirements and retaining, enriching and fortifying the food supply to prevent deficiency diseases. It is now abundantly clear, providing the essential nutrients to humans is necessary but not sufficient to their overall health. Diseases caused by unbalanced diets have become literally epidemic across the developed world (Popkin, 2006; Quam et al., 2006). These conditions include metabolic diseases, atherosclerosis, diabetes, alzheimer's, hypertension and osteoporosis, immunologic diseases, allergy, autoimmunity, immune senescence, and physiological diseases, obesity, hypertension. Food and nutrition research must address these challenges and consider new strategies that can prevent all of these conditions. Humans are sufficiently different that the same diet will not be optimal for all, and some form of personal diet management is necessary. This realization would appear to be devastating to the current system of industrial food production based on large factories producing homogeneous products to high tolerances of safety, quality and cost. Yet the challenges are not insurmountable. Personal health assessment is needed. Foods will need to be customized to more variable compositions. The food marketplace is already highly plastic in many aspects. Consumer products are highly personalized in other sectors and food could adapt rapidly.

At present there is a lack of understanding of how to assemble molecules, biomaterial components, structures much less diets that are preventative of disease rather than curative. Without a model for how to proceed, it is difficult to imagine a path forward. Fortunately milk and lactation provide a myriad of examples to guide research. Lactation emerged through evolution as a unique nourishment system for mammalian infants. The tools of modern biology can now integrate the cellular processes of lactation with the nutritional functions of the molecules and structures that are produced. The evolution of the mammary gland has provided a context in which the development of milk has led to the successful survival of mammalian offspring. Evolution can now guide the next stages of lactation research into understanding diets for life long health.

■ Acknowledgements

Support of the California Dairy Research Foundation, UC Discovery, NIEHS Superfund Grant P42ES04699 and the CHARGE study (PO1ES11269).

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