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Milk Fat Globule structure & function; nanoscece comes to milk production

Nurit Argov^{a,*}, Danielle G Lemay^a, and J Bruce German^{a,b}

^a Department of food Science and Technology, University of California, Davis, 1 Shields Avenue, Davis, CA 95616, United States ^b Nestle Research Center, Lausanne, Switzerland

Abstract

The biological process of fat globule assembly and secretion produces highly complex globule compositions and structures with many properties now recognized to be the direct result of these structures. During homogenization, fat globules are broken down and subsequently structures and surfaces different than the native state are formed. This process alters the milk fat globule unique macrostructure and the effects associated to their structure would be expected to be lost. In the present overview, the need for continued research of the fundamental aspects of the mechanism involved in milk fat globules synthesis secretion and size distribution, as well as establishing ways to regulate those processes are highlighted. Ultimately these insights will guide food technology to developing a new generation of structure based functional foods and as highlighted in this overview, dairy functional products should be the pioneering commodity.

Introduction

Food science is moving from foods developed to simply deliver essential nutrients to foods that promote health of the individual consumer. In the rush to develop this new product category, the major emphasis has been on adding single bioactive ingredients to more traditional foods. However, little attention has been paid to the overall molecular and macromolecular structures in food materials and their importance to the health properties of the foods. Therefore, there is a genuine need to gain knowledge of food structure and the association between structure and function. We have used mammalian milks as genetic, molecular and structural models to understand nourishment using the basic underlying principle of Life Sciences, Darwinian evolution. Milk and all of its components emerged through evolution under the relentless selective pressure to be nourishing. Like any other milk ingredient and structures, the assembly of milk fat globules was evolutionary evolved in order to promote the mammalian infants' health.

The traditional objectives of milk processing are to ensure a long shelf life, to produce satisfactory sensory properties, and to eliminate pathogens. Increasingly, processing strategies have been developed to separate milk components into discrete molecule classes (fat, protein, carbohydrate) to be used as ingredients in a broader range of food applications

*Corresponding author. Department of Food Science and Technology, 1 shields Avenue, University of California Davis, CA 95616, United States. Tel: +1 530 752-1057, FAX: +1 530 752 4759, nargov@ucdavis.edu.

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(Goff & Griffiths, 2006). An important question to ask as these processing methods are developed is what are the milk properties that are lost as a result?

The nutritional value of any commodity can be studied under two perspectives- the delivery of essential components and the presence of non-essential but beneficial structures and activities. The food industry is still challenged by the need to acquire knowledge regarding structures involved in food assembly. This knowledge will eventually be used to develop foods with special characteristics that will deliver maximal health properties to the consumer. From this perspective, milk provides a compelling opportunity to study the nanostructures that self-assemble in milk to maximize mammalian infants' health.

Of special interest are the milk fat globules (Ma & Barbano, 2000; Michalski, Leconte, Briard-Bion, Fauquant, Maubois & Goudedranche, 2006). In addition the recent identification of small, non-triglyceride particles (Argov, Wachsmann-Hogiu, Freeman, Huser, Lebrilla & German, 2008) are of particular interest for their health properties. Therefore the mechanisms controlling the synthesis, assembly and size distribution of the small particles in milk should be studied separately from the large fat delivering particles. Pathways used to facilitate phospholipids discharge such as lamellar bodies (Veldhuizen & Possmayer, 2004) or membrane derived phospholipid exosomes (Blanc, Barres, Bette-Bobillo & Vidal, 2007) should be assessed as possible pathways elaborated by the mammary gland to assemble the milk fat globule macrostructure.

In the USA alone over 30 billion gallons of milk are consumed as liquid milk annually (Goff, *et al.*, 2006). The last 25 years were characterized by significant advances and novel techniques that equip factories to cope with more than 70 billion gallons of milk produced in the USA every year. One of the ways to facilitate milk processing is homogenization. Throughout the last two and a half decades, homogenization improvement has focused on producing the same fat globule size distribution applying lower operation pressure with standard homogenized valves.

Homogenization has little effect on the composition but important effects on milk structure and moreover on milk bioactivities as was previously shown (Michalski, Soares, Lopez, Leconte, Briard & Geloën, 2006). As the most complex entity in milk, the milk fat globules are the macrostructure most affected by homogenization.

In this paper, milk lipid structures are reviewed emphasizing on milk fat globule composition in addition to the health properties related to its consumption. Implications for the manipulation of the milk fat globule by the milk production and processing industry are discussed.

The Importance of Food Structure in Milk

The traditional nutritional view of food as a delivery system for individual nutrients is evolving due to the augmented understanding that diet affects health in more ways than simply through providing essential nutrients. It is now more apparent that food is a multi-component, multi-phasic ensemble of biomaterials that is dynamically altered during the processes of digestion and absorption. Because food is not consumed as individual nutrients, the structure of food influences how nutrient components are digested and absorbed in particular in the temporal dimension. Food matrices with complex structures generally slow the rate of digestion and absorption, a property which may be advantageous or deleterious depending on the biological context. In the context of vitamin bioavailability in an individual who is deficient in that vitamin, a rapid and complete absorption may be favorable. However, in the context of glucose bioavailability in a diabetic individual, a slow and incomplete absorption might be preferred. In both cases, food processors have the

opportunity to create products that influence the health of individual consumers via the manipulation of food structure.

Nowhere is the importance of food structure more evident than in evolution's recipe for the nutrition of mammalian infants: milk. Structural properties of milk regulate the digestion and absorption of each of the major macronutrients—carbohydrates, proteins, and fat. The remainder of this section will explore each in detail.

Glucose is the metabolic fuel of the brain and a central component of metabolic and biosynthetic pathways. Studies have recently shown that the rate of glucose absorption and delivery to the bloodstream affects subsequent endocrine regulation, energy homeostasis and long term health (Barclay, *et al.*, 2008). Milk contains considerable quantities of glucose molecules; yet none is present as simple glucose. In milk, glucose is present as lactose, a disaccharide that can only be cleaved by the enzyme lactase. This allows the rate of glucose delivery to be directly regulated by the lactase-producing consumer of the milk, a process which inevitably slows the delivery of glucose.

Amino acids are the required building blocks of proteins and a necessary natural supply for all mammals especially growing infants. Amino acids are provided in milk mainly via casein proteins. Generally, the greater the degree of protein structure, the more resistant the protein is to proteases and the more difficult the digestion. Because casein proteins have little secondary or tertiary structure, they are effectively built for rapid, easy digestion. In vitro studies of casein proteins confirm that they are highly susceptible to proteolysis (Baglieri, Mahe, Benamouzig, Savoie & Tome, 1995). However, the protein structure of milk has an additional layer of complexity to regulate the speed at which these easily digested proteins are made available to the infant gut. Casein proteins aggregate to form micelles that are soluble in water. In the infant stomach, chymosin cleaves a surface-stabilizing peptide from the kappa-casein molecule and enables a self assembly process of the casein micelles into insoluble curds. The curds are released in turn more slowly to the intestinal lumen. This system suggests that the easily digested casein proteins are delivered to the infant's small intestine in a sustained release manner to slow the speed of digestion while maintaining high digestibility.

The lipid fraction within milk was oversimplified by previous research (Timmen & Patton, 1988) by referring to this fraction as relatively pure triacylglycerols (TG) and disregarding the unique and complex macrostructures inherent in this unusual biological colloidal system. Actually, this unique macrostructure and its biochemical assembly process distinguish the milk fat globule from both simple plant derived oils and other animal fat systems. While milk carbohydrates and proteins structures were extensively studied and their applications in digestion and absorbance rates were described, such studies for the lipid entities within milk have not been conducted. Lipids are provided in milk via a unique delivery system: milk fat globules. Prior to the evolution of mammals, insects, reptiles, fish, and amphibians had already elaborated a highly functional system of lipoproteins for the movement of lipids through aqueous biofluids (e.g. blood; Fig. 1). Lipoproteins consist of a single layer of amphipathic phospholipid and cholesterol molecules that surround the non-polar lipids (Siri & Krauss, 2005). Given this simple established system available early in animal evolution, it is surprising that fats from milk are not transferred in the same manner. Rather, fat transfer in milk is much more resource intensive because portions of the mammary epithelial cell membrane are sacrificed to secrete the milk fat globules. This unique food structure for the delivery of lipid is the focus of the remainder of this article.

Milk Fat Globules as Products of Evolution

Milk is the only whole food which was evolved in order to completely meet the nutritional needs of the respective suckling mammalian neonates. This early nutritional strategy of mammals produced a remarkable Darwinian engine for driving the nutritional values of milk according to their abilities to maximize the survival chances of infants (German & Dillard, 2006). Any components or structures added to milk cost the resources of the mother and hence would place her at a selective disadvantage. They are only of value then, to the extent that they protect her or encourage the success of her infant. Hence, why should the mammary gland elaborate such a unique bioprocess (Mather & Keenan, 1998) and highly energy consuming pathways to synthesize, assemble and secrete lipids into milk?

To understand the uniqueness of the structure and the forces driving the emergence of such an exclusive particle the milk fat globule should be studied under a broader perspective than the common approach. The intuitive notion that milk fat globules only role is to deliver fat is conspicuously wide of the mark since at least in some lactation stages, large portion of the lipid globules by number are in the submicron length scale (Michalski, Briard, Michel, Tasson & Poulain, 2005) and therefore are relatively triacylglycerol poor.

Lipid related research up to date has focused on understanding and managing to alter mainly fatty acid composition and concentration of milk lipid fraction which was found to be highly associated with the mother's dietary fatty acid composition (German, *et al.*, 2006). Nonetheless, very few studies looked at the possibility that these alterations might modify the milk fat globules (MFG) and milk fat globular membrane (MFGM), and almost none examined what industrial and subsequent nutritional impact such change, might have.

If indeed the dietary change influences the milk lipid macrostructure, it is not clear what outcomes those alterations have on different nutritional and digestion parameters. Will it enhance lipid digestion by gastrointestinal enzymes? Will it improve absorption by intestinal entocytes? it might have implications on the lipid and fat absorption rate and subsequently on lipid metabolism. The MFG secretion mechanism evolved in order to optimize the lipid delivery to the suckling mammal by means of nutritional value. Thus the MFG macrostructure should be studied in order to further understand its role in enabling advantageous absorption (not necessarily rapid and complete absorption) and optimizing succeeding lipid tissue distribution. It should be recalled that the Darwinian selective pressure was exerted on the mammary gland and milk thousands of years before heart disease became the main epidemiological health issue in the western word. Therefore, milk lipid macrostructure might affect health issues that are currently not assessed with respect to fat.

Milk Fat Globule Composition, Synthesis, and Size Distribution

The lipid composition of a MFG is traditionally considered to be solely a function of the length and degree of unsaturation of the fatty acids. Fatty acids tend to be esterified to different lipid fractions depending on their carbon chain length and degree of unsaturation (Prieto, DePeters, Robinson, Santos, Pareas & Taylor, 2003). In addition, milk fat globules span a wide range of sizes with quite different size classes having different TG/PL composition. Hence, differences in the TG/PL ratios in the MFGs of different diameters also indicate different FA compositions. The composition of the membrane phospholipids and the fatty-acid profile within the phospholipid fraction certainly play important roles in the milk fat globule membrane's physical and functional properties. Nonetheless, our ability to control membrane properties and the processes of milk fat globule assembly, need to extend beyond simply fatty acid composition.

The MFGs triglycerides are synthesized at the rough endoplasmic reticulum, accumulate into triglyceride-rich domains varied in size, which are released as discrete droplets to the cytoplasm, coated with polar lipids and proteins derived from the ER (Mather, *et al.*, 1998). From their site of origin, lipid droplets migrate to the apical pole of the cell from which they will be secreted. Milk fat globules follow a distinct export process in which the plasma membrane itself enrobes the emerging globule (Wooding, 1971). In the secreted milk, lipid globule diameters range from 0.2 to more than 15 μm (Michalski, Cariou, Michel & Garnier, 2002) which implies that some lipid droplets grow substantially between the time of their formation and the time of their secretion (Scow, Blanchette-Mackie & Smith, 1980). Furthermore, post-secretion, some of the larger milk fat globules may fuse with smaller ones and by that increase their diameter. However, whether this process is explicitly directed by cellular processes or whether the globules spontaneously self-assemble according to the composition of the respective surfaces, is unknown. The fact that milk lipid fraction, consist of MFG in various diameters (Bauman, Mather, Wall & Lock, 2006) implies that their size distribution within milk may have additional role than simply deliver fat to the infant. Moreover the diameter distribution might be altered under different physiological conditions since it has been shown to change in different lactation stages (Michalski, *et al.*, 2005) and subjected to nutrition administrations (Couvreur, Hurtaud, Lopez, Delaby & Peyraud, 2006). Because MFG synthesis and secretion pathway is unique, major studies were conducted during the last two decades, pursuing ways to understand the MFG biological mechanisms enabling their synthesis and secretion. However the intracellular mechanisms remain illusive. If the basic mechanisms of the processes that dictate globule size distribution were known, and if indeed the nutritional importance is established, they could be controlled, and the size of particles, the compositions of surfaces and net composition of milk lipids (Fig. 2) be explicitly guided to desired goals.

The most studied lipid of the entire cream fraction of milk is the triacylglycerol core of the droplets which typically consist of more than 99% of milk lipids (Patton & Jensen, 1975). Nonetheless, this overwhelming predominance of neutral lipids obscures the complexity of the processes, structures and functions of actual milk fat particles. Once those globules are secreted into the milk they are completely coated with the cellular bilayer membranes of the epithelial cells of the mammary gland. (Patton, *et al.*, 1975). Therefore all the apical membrane physical, chemical and functional features are carried over to milk by the milk fat globule membrane. Although extensive proteomics studies were conducted in order to characterize the proteins in the MFGM (Reinhardt & Lippolis, 2006) it seems that a parallel lipidomic study has not yet been conducted. In the nutritional, as well as industrial point of view, this kind of research might be highly valuable. Because protein distribution is dictated by genes, the ability to alter their distribution within milk is somewhat restricted. However, the lipid fraction of the milk can be modified nutritionally (Palmquist, Beaulieu & Barbano, 1993) Therefore in order to enable control of the milk lipid macrostructure, further understanding of the regulating factors as well as the bioactivities of the macrostructure, is required.

Health Properties of Milk Lipid Macrostructure

As was previously reviewed (Michalski, 2007) milk fat globules in their native macromolecular structures have a wide range of speculated health benefits. Since technologies necessary to describe more complex lipid structures in smaller length scales are being applied in other lipid research fields (i.e. lipoproteins)(Tian & Jonas, 2002), those same techniques should be applied to characteriznig the smallest milk fat globules.

Researchers have recently investigated whether the source of dietary fatty acids, mostly long chain poly-unsaturated fatty acids (PUFA) such as EPA, DHA and AA, influences the

regulation of their incorporation into tissues and erythrocyte membranes (Sala-Vila, *et al.*, 2006). Whether the chemical form of PUFA administration affects its bioavailability is unresolved (Alessandri, Goustard, Guesnet & Durand, 1998; Amate, Gil & Ramirez, 2001; Goustard-Langelier, Guesnet, Durand, Antoine & Alessandri, 1999; Sala-Vila, Castellote, Campoy, Rivero, Rodriguez-Palmero & Lopez-Sabater, 2004; Wijendran, Huang, Diau, Boehm, Nathanielsz & Brenna, 2002). The natural delivery of milk fatty acids is the MFG where the PUFA are principally attached to the polar head group of phospholipids (Prieto, *et al.*, 2003) that form the surface layers of the MFGM (Jensen, 1999). Thus, the possibility that MFG structure has a role of delivering bioactive advantageous nutrients, and moreover, essential compounds to the suckling neonate, should be considered when the bioavailability of the PUFA is examined (Fig. 3).

Plasma lipoprotein distributions, cholesterol distribution within different lipoprotein classes, and the composition of various lipids within the lipoproteins of adults and children are influenced by normal or specifically enriched milk consumption (Benito, *et al.*, 2006; Kohn, Sawatzki, van Biervliet & Rosseneu, 1994; Tricon, *et al.*, 2006). Discouragingly, relatively few studies have examined the role of milk in altering infant lipoprotein distributions, even human breast milk. Nonetheless, those reports that have emerged have indicated that breast feeding versus formula feeding does influence the distribution of lipoproteins and by inference alters the overall lipid metabolism of infants (Hayes, Pronczuk, Wood & Guy, 1992; Nelson & Innis, 1999). The obvious fact is that the unique lipid macrostructure within milk is exceedingly difficult to replicate or imitate. Therefore, while most abundant milk proteins and carbohydrates (i.e. caseins and lactose) are found in both milk and infants' formula, the lipid structure factor is found only in milk. Consequently, this unique structure should be considered when whole body lipid metabolism is evaluated after milk and various dairy products are consumed.

The ability of intestinal epithelial cells to synthesize a variety of apoproteins (Black, 2007) and assemble and secrete lipoproteins has been extensively studied. Subsequent to lipid digestion, triacylglycerols are hydrolyzed within the intestinal lumen to form free fatty acids, which diffuse across the apical membrane of enterocytes. In the enterocytes, fatty acids re-esterify to form TG, PL and cholesterol- esters (Ockner & Manning, 1974). Two distinct pathways by which fatty acids are incorporated into TG have been described (Mansbach & Parthasarathy, 1982) The main variation between those two pathways is the transport rate of re-esterified TG to the intestinal mucosa and the liver. The possibility that the rate of TG efflux from the digestive tract to the rest of the body tissues could be nutritionally regulated by up/downregulating each of the above pathways, is not new (Kalogeris, Gray, Yeh & Tso, 1996; Mansbach, Arnold & Cox, 1985). In this context, the milk fat globule native structure and the different sizes of the globules might be differently affected by processes in the infants' gastrointestinal tract as well as subsequent digestion and absorbance rates. Therefore, we hypothesize that under different circumstances in which different nutrient delivery rates are valuable, milk fat globule production and their size distribution are subjected to the lactating mother's physiological state.

Industrial Applications

The milk lipid fraction is known to contribute to the textural characteristics of many dairy products (Couvreur, *et al.*, 2006; Michalski, *et al.*, 2002). Lipid globules of different diameters exhibit different thermal and physiological properties (Michalski, Ollivon, Briard, Leconte & Lopez, 2004) and different lipid compositions (Michalski, *et al.*, 2005). In order to reduce the size distribution of MFG industrially, technologies have been developed to disrupt the native structure of globules, most commonly by homogenization (Lopez, 2005). Homogenization is achieved by artificially increasing the number and net surface area of

MFGs. At the end of the homogenization process, the large amount of the new milk fat globules surface area created by homogenization must be covered by protein. Cano Ruiz and Richter estimated that only of 10% of the total MFGM after homogenization is covered by the original MFGM material (Cano-Ruiz & Richter, 1997). The newly formed fat globules exhibit different physical and chemical properties (Fig. 2). In addition, the MFG special structure is disrupted and interactions between different milk constituents like caseins and whey proteins occur, which in turn will influence the dairy products properties (Michalski, *et al.*, 2002). Therefore, the development of adequate protocols and nondestructive methods which will facilitate naturally MFG usage would enable a new dimension of milk functionality to be explored, leading to dairy products with altered functional, nutritional and physical properties.

From a nutritional prospective, It has been previously shown that some fractions of the MFGM may be shed into the skim milk (Singh, 2006) and spontaneously assemble into liposomes, or those liposomes might be artificially formed due to homogenization. Through disruption of the native macrostructure by homogenization, some bioactivities or at least bioactive molecules might be carried over to the skim milk. Hence, the possible effects on digestion, absorption and plasma lipid distribution should be assessed in relation to the small milk fat globule macrostructure and skim milk liposomes.

Due to the nature of the biological processes of MFG assembly combined with its physio-chemical properties of native milk fat globules that vary according to their size, it is possible to imagine producing milks with highly controlled and nonetheless highly native MFG's.

Conclusion

The milk fat globule remains the least understood component of one of the world's most valuable agricultural commodities, milk. Darwinian selective pressure drove the emergence of a remarkable lipid delivery system in which the particles and their surface properties are unique to any other biological lipid export system. Recent nutrition research is identifying multiple factors associated with the milk fat globule membrane and distinct health properties. Unfortunately, there are no available data regarding the outcome of the genetic, screening and breeding in bovine milk in the past 100 years on the milk lipid macrostructures. We propose to understand the key biochemical and physical steps associated with fat globule formation and secretion with the explicit goal to control them. With this basic knowledge in place we shall then be able to manipulate the size, abundance and very importantly the amount and composition of the surfaces of these particles. One obvious application of this research would be to produce milk naturally enriched with small globules. Bringing to practice such a bioguided process of milk homogenization would explicitly increase the number and surface of MFGs and preserve their native structure.

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Fig. 1.

Throughout evolution organisms developed the ability to secrete lipoproteins to enable lipid movement in aquatic solutions. The macrostructure of triglyceride core enveloped by triple-layered phospholipids coat is uniquely found in mammals' mammary gland secreted solution- milk.

Fig. 2.

Milk fat globules size, volume and surface area parameters in native and homogenized milk compared with lipoproteins. Distribution plots indicated size distribution by number of the milk fat globules as was found in human breast milk and bovine homogenized milk.

Fig 3. Nutritional implications, health properties and the structural function of the bioactive molecules carried over by native milk fat globules macrostructure.