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BIOFUNCTIONAL PROPERTIES OF MILK FAT GLOBULE MEMBRANE PHOSPHOLIPIDS

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Abstract

The aim of the paper is to discuss the general properties of polar lipids present in the milk fat globule membrane (MFGM), their role in living organism and some benefits of consuming these components in the human diet.

Milk phospholipids contain glycerophospholipids and sphingolipids. These compounds perform a multitude of functions in living organisms: they serve as energy storage for the body, are a building blocks of cell membranes, they and their derivatives are bioactive molecules involved in the mediation and recognition of signals, and interact with other cell components. There has been an increasing number of reports documenting the health benefits of milk lipid consumption. Although they are present in milk in small quantities, their unique properties may help prevent and alleviate numerous diseases.

Introduction

There has been an increasing number of reports indicating that breast-feeding is vital for proper infant development (DONOVAN 2006, LEE et al. 2018). It also benefits the mother's health by reducing the risk of breast and ovarian cancers (JONES 2007). This synergistic interaction is the result of an evolutionary adaptation through which mechanisms advantageous for survival have emerged. The effects of milk on the child's health are extremely complex, and providing the infant with essential nutrients is only part of milk's functions. As such, milk continues to serve as a model food-stuff, while researches also draw upon it as an inspiration and a source of knowledge on the two-way links between humans and nutrition. Advancing

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the knowledge on the biological functions of milk constituents will help better harness the prospective properties of nutrients to develop biofunctional foods with distinct therapeutic values, designed to combat specific diseases or satisfy specific consumer needs.

Though scarce in the membrane, polar lipids play an important role in nutrition and normal cell function. Due to their unique properties, polar lipids perform numerous functions in living organisms: they serve as energy storage for the body, are a building block of cell membranes (isolating the cell from the environment and separating cellular compartments), they and their derivatives are bioactive molecules involved in the mediation and recognition of signals, they also interact with other cell components (NICOLSON and ASH 2014).

There has been an increasing number of reports documenting the health benefits of milk lipids in general, and the biological role of phospholipids in particular (AMBROZIAK and CICHOSZ 2013, SMOCZYŃSKI 2017, KUCHTA et al. 2012, EL-LOLY 2011). Consequently, the aim of the present paper is to discuss the general properties of polar lipids present in the milk fat globule membrane (MFGM), their role in living organism and some benefits of consuming these components in the human diet.

Lipid components of the milk fat globule membrane (MFGM)

Milk lipids are likely the most complex and least understood group of milk constituents. Lipid content depends on the species and ranges from 3.8 to 3.9% for woman's milk, and 3 to 5% for bovine milk. In mammal bodies, the lipids are formed in the epithelial cells of the mammary gland and secreted in emulsified form as milk fat globules coated with a phospholipid/protein trilayer. Secretion of fat globules is a process unique to the mammary gland and has been a subject of many studies (HEID and KEENAN 2005, MATHER and KEENAN 1998). In structural terms, fat globules consist of an apolar inner core mostly composed of triacylglycerols and cholesterol esters, surrounded by a membrane about 10–20 nm in diameter. This milk fat globule membrane has a very defined structure, making up 2–6% of the total globule mass, and consists mainly of proteins and phospholipids. The content of phospholipids in the membrane is estimated at 40 to 75% (DEETH 1997, SINGH 2006).

Milk phospholipids contain glycerophospholipids and sphingolipids. Glycerophospholipids consist of a glycerol backbone esterified with fatty acids at two positions, and a phosphate with a different organic group

(e.g. serine, choline) at the third position. For sphingolipids, the structure varies and is based on a sphingosine, i.e., a long-chain aliphatic amine (which additionally contains two or three hydroxyl groups). The attachment of a fatty acid to the amine group results in the formation of ceramide. If phosphocholine is attached to the ceramide, sphingomyelin is formed, whereas the attachment of a saccharide group produces a sphingoglycolipid (DEWETTINCK et al. 2008, VESPER et al. 1999).

Phospholipids account for up to 1% of milk fat (SÁNCHEZ-JUANES et al. 2009). In mammal cells, the majority of such lipids are produced in the endoplasmic reticulum (VANCE 2018). Table 1 presents the composition and content of the main lipid components of the milk fat globule membrane (based on FONG et al. 2007, SINGH 2006, SMOCZYŃSKI et al. 2012). Although the main fraction are triacylglycerols, the content of which varies in the literature between 20 to 80% of the membrane weight, the majority of triacylglycerols can originate from the contamination by the core of the fat globules during isolation of the membrane. Therefore, the literature shows a wide variation depending on the methods used to isolate the material (WALSTRA 1985).

Table 1

Lipid components of the milk fat globule membrane (FONG 2007, SINGH 2006)

Lipid group	Percentage [%]
Triacylglycerols	56.0–62.0
Diacylglycerols	2.1–9.0
Monoacylglycerols	0.4
Free fatty acids	0.6–6.0
Sterols	0.2–2.0
Phospholipids	26.0–40.6
including [% of phospholipids]:	
Sphingomyelin	20–22
Phosphatidylcholine	31–36
Phosphatidylethanolamine	27–30
Phosphatidylinositol	7–11
Phosphatidylserine	4–5
Lysophospatidylcholine	2
Lactocerebroside	3.4
Glucocerebroside	0.3

Characteristics of MFGM polar lipids

Phosphatidylcholine

Phosphatidylcholine (PC, also referred to as lecithin) is a phosphoglyceride in which the phosphate group is esterified with choline, and makes up 40 to 50% of the cell membrane phospholipids. PC is a zwitterion

over a wide range of pH values, owing to the presence of a positively-charged quaternary amine group and a negatively-charged phosphate residue. PC is biosynthesized in the endoplasmic reticulum (ER) through the so-called Kennedy pathway (KENNEDY and WEISS 1956), first by phosphorylating choline to form phosphocholine, then incorporating sn-1,2-diacylglycerol into the latter. The structure of PC is subject to changes through the exchange of fatty acids within the molecule, a process known as the Lands cycle (LANDS 1958). In the liver, phosphatidylcholine can also be synthesized through methylation of phosphatidylethanolamine (VANCE and RIDGWAY 1988).

Other PC constituents include: palmitic acid, stearic acid, oleic acid, linolenic acid, and arachidonic acid, with the saturated and unsaturated acids mostly occurring in the sn-1 and sn-2 positions, respectively. PC can be degraded by phospholipases, which exhibit specificity for the cleft bonds and thus can release certain metabolites important to signaling. The released choline is re-incorporated into new PC molecules (KRAHMER et al. 2011).

PC is involved in a number of important functions in the body. Together with other phospholipids, it plays a major role in lipid metabolism, being involved in the synthesis and transport of lipid-derived lipoproteins within the body. In combination with proteins and cholesterol, it forms a single-layer surrounding the hydrophobic core of lipoprotein particle composed of triacylglycerols and cholesterol esters. The essential role of PC in maintaining normal function of lipoproteins also relates to the presence of polyunsaturated carboxylic acids at the sn-2 position. The changes in the PC molecule brought about by the Lands cycle enable compounds, such as arachidonic acid, to be incorporated into the sn-2 position, modifying the biomembrane fluidity in turn (RAWICZ et al. 2000). Lower proportions of arachidonic acid in PC molecules have been shown to inhibit the synthesis and release of VLDLs (very low-density lipoproteins), leading to triacylglycerol accumulation in the liver (RONG et al. 2015). PC also indirectly regulates the synthesis and dynamics of intracytoplasmic lipid droplets. Suppressed PC biosynthesis in adipocytes leads to increased droplet size (KRAHMER et al. 2011).

Phosphatidylethanolamine

After PC, phosphatidylethanolamine (PE) is the second most abundant phospholipid in milk fat globule membranes and plant/animal cell membranes. It is mainly biosynthesized through the incorporation of cytidine diphosphate-ethanolamine into diglyceride – releasing cytidine

5`-monophosphate – or through the decarboxylation of phosphatidylserine (GIBELLINI and SMITH 2010).

Arachidonic and docosahexaenoic acids are major constituents of the PE, attached mainly at the sn-2 position, whereas saturated acids are the most common component at sn-1. In some types of cells (neurons, cells involved in the inflammatory response), a large proportion of PE (more than 50%) occurs as plasmalogen, i.e., the form containing an ether bond. By contrast, the plasmalogen form of PE is in trace amounts in the liver (HAN et al. 2001).

Unlike PC, PE – together with phosphatidylserine – occurs mainly in the inner part of the lipid bilayer, from the cell interior side. Being a polar head group, PE can form hydrogen bonds with other lipids or proteins, which stabilizes proteins within the membrane (YEAGLE 2014). The small size of the polar head can alter the curvature of the membrane surface and change the fluidity of the biomembrane (DAWALIBY et al. 2016).

PE also plays a major role in lipid metabolism and the formation of cytoplasmic lipid droplets. It is particularly associated with the function and metabolism of VLDL molecules – its presence in these molecules promotes their rapid take-up and removal from circulation. This may result in the lower risk of hypercholesterolemia (VEEN et al. 2017). Biomembranes also contain smaller quantities of phosphatidylcholine derivatives, such as lysophosphatidylethanolamine – which bears only one acyl residue – or mono-/dimethyl-phosphatidylethanolamine (GIBELLINI and SMITH 2010).

Sphingomyelin

Sphingomyelin (SM) is a sphingolipid, a complex group of lipid compounds, in which the long-chain amino alcohol sphingosine comprises the backbone. Sphingomyelin is formed through the binding of a fatty acid residue with an amide bond, after which a choline phosphate group is attached to the resulting ceramide. The biosynthesis and metabolism of sphingolipids are strictly controlled, influenced by multiple factors, and include many intermediate metabolites with their respective biological activities. By contrast, SM is predominantly formed by transferring phosphocholine from PC onto the ceramide (MERRILL 2011). The group was named in reference to the sphinx, which was intended to evoke their heretofore enigmatic properties. However, the biological role of its compounds has been increasingly documented in scientific literature (MERRILL 2011).

Like PC, SM is a zwitterion. It can account for up to half of the membrane lipids in some tissues, but in most cases PC exceeds it in proportion. Also, like PC, SM occurs mainly in the outer layer of the plasma mem-

brane. The SM molecule predominantly contains long-chain saturated fatty acids (palmitic and stearic) with a small number of monounsaturated fatty acids (RAMSTEDT et al. 1999).

Sphingolipids, including sphingomyelin (SM), are involved in a number of important functions in the body. One of the main functions of SM – resulting from its unique structure – is combining with cholesterol to form domains known as lipid rafts, i.e., special areas on the surface of the membrane that are slightly tighter packed than the others (SIMONS and IKONEN 1997). These domains serve as interaction sites for certain proteins. Since SM content correlates with cholesterol levels in membranes, SM can affect the levels and metabolism of cholesterol (VESPER et al. 1999). Moreover, SM is a major source of ceramides and other metabolites that act as signal transducers for growth regulation, cell migration, adhesion, apoptosis, and inflammatory response (HANNUN and OBEID 2018).

Phosphatidylinositol

Phosphatidylinositol (PI) is a glycerophospholipid that contains carboxyl acid in ester linkage with glycerol, as well as inositol (hexahydroxycyclohexane) bonded via a phosphoric acid residue. The PI molecule is negatively charged. This phospholipid has the unique characteristic of having high levels of stearic and arachidonic acids at sn-1 and sn-2, respectively (D'SOUZA and EPAND 2014). Biosynthesis of phosphatidylinositol is mediated by phosphatidylinositol synthase, which catalyzes the bond formation between CDP-diacylglycerol and inositol.

Apart from being a constituent of biomembranes, PI also facilitates the anchoring of various proteins to the membrane, while its metabolites also play a major role in signaling. The activity of phospholipase A_2 can stimulate the release of arachidonic acid, a main substrate in the biosynthesis of eicosanoids, such as prostaglandins, whereas phospholipase C activates the release of diacylglycerol. Diacylglycerol, in turn, regulates the entire enzyme family referred to as protein kinase C, a signaling pathway involved in a multitude of cell processes, including differentiation, proliferation, metabolism, and programmed cell death (DE CRAENE et al. 2017).

Phosphatidylserine

Phosphatidylserine (PS) is an essential bioactive anionic glycerophospholipid, containing two fatty acid residues, as well as serine attached to a phosphoric acid residue.

In animals, PS is biosynthesized through a calcium-dependent reaction, in which the polar head-group of a pre-existing phospholipid (PC or PE) is exchanged. A portion of the newly-synthesized phosphatidylserine, after transfer to the mitochondrion, can be decarboxylated, producing PE. Similar to other phospholipids, the fatty acid composition of PS can be modified through the Lands cycle (LANDS 1958, VANCE 2018). PS is usually present at low levels, though its concentration may be as high as 20% of the total phospholipids in plasma membranes and the brain (VANCE 2018).

As an active phospholipid, PS plays an important role in multiple signaling pathways. Since the PS molecule is charged, it greatly affects cell membrane structure, as well as the incorporation of certain proteins and ions (PS is mostly present on the cytoplasm leaflet of the bilayer) (VANCE 2018). These interactions and the incorporation of specific proteins or enzymes, often via calcium channels, are essential to specific activity and normal signaling. It is through these mechanisms that PS is directly involved in brain signaling, incorporating signal transduction proteins in neurons, and thus activating them (KIM et al. 2014).

PS is a key regulator of apoptosis. Increased concentration of Ca^{2+} activates scramblase, an enzyme that translocates PS to the outer leaflet of the lipid bilayer. Cell surface exposure of PS most likely enables immune system cells (e.g., macrophages) to bind onto a damaged cell through the proper receptors and remove it (BEVERS and WILLIAMSON 2016). PS also plays a major role in blood clotting by stimulating the activation of prothrombin to thrombin (LENTZ 2003).

Other phospholipids

Milk fat globule membranes also include other phospholipids and their derivatives, among them lysophosphatidylcholine, lactocerebroside, and glucocerebroside.

Lysophosphatidylcholine (LPC) can be produced from PC during digestion and absorbed. It can also be synthesized from PC in the body by phospholipase A_2. Lysophosphatidylcholine contains docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and as such can deliver these acids to the brain using specific transport mechanisms (LAW et al. 2019). LPC may also exhibit antibacterial effects (RENSBURG et al. 1992).

Glucocerebroside (GluCer) and lactocerebroside (LacCer) are both sphingolipids. LacCer is produced when a galactose molecule (from the active form of UDP-galactose) is bonded onto the precursor (GluCer) by the β-galactosyltransferase enzyme. These phospholipids are important intermediates in the biosynthesis of more complex glycolipids, such as oli-

goglycosylcerebrosides or gangliosides, which incorporate additional carbohydrate residues into their structure (LINGWOOD 2011).

Glycolipids play an important role in the immune response and may induce the stabilization of lipid rafts. Glycolipids present on macrophage surfaces are involved in interactions between carbohydrate groups, facilitating the binding of bacteria and fungi and their subsequent elimination (LINGWOOD 2011). When present on the outer leaflet of the lipid bilayer, they form a structure known as a glycocalyx – a membrane coating covered with carbohydrate residues. This coating serves a protective function, but also plays a major role in intercellular communication, targeting of pathogens, and modulation of the inflammatory response (D`ANGELO et al. 2013).

Nutritional aspects of milk polar lipids

There is increasing number of scientific literature considering the health-promoting properties of the milk fat globule membrane components (TOKAS 2019). Many reports indicate a direct relationship between the consumption of membrane components, including polar lipids, and disease. Phospholipids and sphingolipids show a broad spectrum of activities as functional ingredients due to their regulatory properties, in addition to their structural functionalities. They are also effective at low concentrations (SCHMELZ 2000). The biological activity of phospholipids and their metabolites relates to such interactions as anti-carcinogenic, cognitive development, neurological diseases and aging (e.g. Parkinson, Alzheimer), reduction of cardiovascular risk and inflammation, liver recovery, neonatal gut development and gastroprotective role (DEWETTINCK et al. 2008, RODRIGUEZ-ALCALA et al. 2017, NICOLSON and ASH 2014).

DILLEHAY et al. (1994) treated mice with 1,2-dimethylhydrazine to induce colon cancer and showed that mice fed diets supplemented with milk sphingomyelin had a lower (20%) incidence of colon tumours compared with 47% in controls. These results showed that consumption of sphingomyelin affects the behaviour of colonic cells. In another study ZHANG et al. (2008) showed that dietary sphingomyelin inhibited the tumorigenesis and increased alkaline sphingomyelinase (a key enzyme responsible for sphingomyelin digestion in the gut) activity in the colon by 65%. SCHMELZ et al. (1996) reported that the administration of isolated dairy sphingomyelin in diet of CF1 mice, transformed malignant adenocarcinoma to benign adenoma.

CASTRO-GOMEZ et al (2016) investigated the in vitro effect of a concentrate of phospho- and sphingolipids obtained from buttermilk. The research

work showed antiproliferative effect on ovary cancer lines, which could be explained by the presence of sphingomyelin in the extract.

In the human body especially high concentration of phospholipids occurs in the brain, where together with long-chained polyunsaturated omega-3 fatty acids constitute the basic building blocks of the nervous system. So many research work relates to phospholipid role in the development of age-related diseases and Alzheimer's disease (SPITSBERG 2005). There are multiple studies indicating that phosphatidylserine supplementation may positively affect brain function (memory), improve health, slow ageing, and produce beneficial effects in Alzheimer's patients (DEWETTINCK et al. 2008, HASHIOKA et al. 2004). Short-term supplementation with PS was also shown to improve exercise capacity during high-intensity cycling, which might suggest an innovative application as a supplement (KINGSLEY 2006).

Phosphatidylcholine is essential for normal functioning of mitochondria, as well as protecting them against reactive oxygen species produced during ATP generation (HAILEY et al. 2010). In rodent studies, it has been proven that the introduction to the diet of milk-derived phospholipids has positive acting in the treatment of steatosis and liver enlargement (WAT et al. 2009). In addition, PC supports the regeneration of alcohol-damaged liver (KHARBANDA et al. 2006), and protects gastrointestinal cells from the harmful effects of toxins (ANAND et al. 1999).

Many studies have shown that consuming sphingomyelin, in addition to its anti-cancer effect, can have beneficial effects, including the regulation of cholesterol levels and protection against bacterial infections and mycotoxins (VESPER et al. 1999).

Dietary lipid supplementation is recommended to improve health by modifying and restoring the composition of cellular and intracellular (mainly mitochondrial) membranes (NICOLSON and ASH 2014). Defects in cell and intracellular membranes are common to all chronic conditions, including cancer and normal processes such as aging. Oxidative stress occurs when the production of reactive oxygen species (superoxide, hydroxyl radicals or hydrogen peroxide) is in excess to the cell's ability to destroy these molecules with its natural antioxidants (HALLIWELL 2006). Cellular targets of this reactive species can be nucleic acids, proteins and also lipids, and mitochondrial structures are especially sensitive to oxidative damage (WEI and LEE 2002). So phospholipid supplementation can have a number of positive effects and has been successfully used in many chronic diseases, without any toxic effects for human (NICOLSON and ASH 2014).

Milk and milk products can be a valuable source of bioactive membrane lipids (Table 2). Raw milk has a polar lipid content of 9.4–40 mg/100 g of milk (ROMBAUT and DEWETTINCK 2006). They are mainly associated with the milk fat globule membrane. Their content in various dairy products is associated with the fat content of the product, so the high-fat products are rich in phospholipids. The most polar lipids are concentrated in the butterserum, but the technological usefulness of this product for obtaining polar lipids is limited. Butter can be considered the best source of phospholipids, due to its high fat content. However, during churning, the membrane of milk fat globule is broken and largely migrates to the aqueous phase, partly due to their high affinity for proteins (YEAGLE 2014, AMBROZIAK and CICHOSZ 2013). So buttermilk is a low-fat product with high concentration of phospholipids. Considering the content of phospholipids in relation to the dry mass, buttermilk contains 4–5 times more than other products (ROMBAUT et al. 2005, AMBROZIAK and CICHOSZ 2013). Taking this into account, buttermilk can be considered as an interesting source for further purification and concentration of phospholipids to obtain a product with high functional and nutritional value.

Table 2

Polar lipid content of some dairy products (PIMENTEL et al. 2016, ROMBAUT and DEWETTINCK 2006)

Product	mg/100 g of product	g/100 g of fat
Raw milk	9–40	0.7–0.9
Cream	139–190	0.3–0.9
Butter	70–230	0.1–0.3
Buttermilk	9–160	4.5–33.1
Butterserum	660–1250	14.8–48.4
Yoghurt skimmed	18	5.5
Kefir semiskimmed	34	2.3
Ricotta	279	2.7
Cottage cheese	56–376	1.3–5.3
Cheddar	154	0.5
Emmental	110	0.4

Conclusion

The present paper describes the main groups of polar lipids found in milk. Polar lipids are not only constituents of milk fat globule membranes, but also major components of biomembranes. They serve as a barrier that isolates cell's interior from the environment – any change or damage to the structure of these lipids, caused by external factors, is the primary means of signaling a threat to the entire cell. From these basic functions, cells have evolved a variety of mechanisms of intercellular and intracellular

signaling, with specific functions activated based on the level of polar lipids and their metabolites.

There has been an increasing number of reports documenting the health benefits of milk lipid consumption. Although they are present in milk in small quantities, their unique properties may help prevent and alleviate numerous diseases. Due to their amphiphilic properties, they play a major role in lipid metabolism and may prove useful in the prophylaxis and treatment of various diseases of affluence.

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