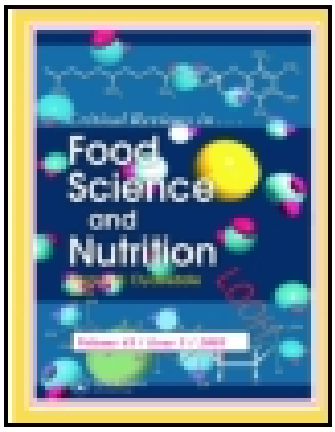

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Phospholipids in Human Milk and Infant Formulas: Benefits and Needs for Correct Infant Nutrition

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Phospholipids in human milk and infant formulas: benefits and needs for correct infant nutrition

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Abstract

The composition of human milk has served as a basis for the development of infant formulas, which are used when breastfeeding is not possible. Among the human milk nutrients, 50% of the total energetic value corresponds to fat, with a high level of fatty acids and 0.2-2.0% present in the form of phospholipids (PLs). The PL contents and fatty acid distribution in PL species have been investigated as bioactive elements for the production of infant formulas, since they offer potential benefits for the optimum growth and health of the newborn infant. The differences in the amount of PLs and in fatty acid distribution in PL species between human milk and infant formulas can imply biologically significant differences for newborn infants fed with infant formulas versus human milk - mainly due to the greater proportion of sphingomyelin with respect to phosphatidylcholine in infant formulas. The limited information referred to the characterization of fatty acid distribution in PL species in infant formulas or in ingredients used

to enrich them merits further research in order to obtain products with benefits similar to those of human milk in terms of infant growth, visual acuity and neurological development. The present review establishes the scientific basis for helping to adjust formulations to the requirements of infant nutrition.

Keywords: Breastfeeding, phospholipids, functional bioactive ingredients, milk composition, neurological development, choline.

1. Introduction

Human milk is regarded as the optimum food during the first stages of life: besides satisfying the nutritional requirements of the infant, it is able to provide additional beneficial effects such as protection against infections or a lesser risk of later allergies. However, when breastfeeding is not possible, use is made of infant formulas with macronutrient and micronutrient compositions as similar as possible to those of human milk.

Among the different human milk nutrients, fat accounts for about 50% of the total energetic value (Jansson et al., 1981), providing essential nutrients, as well as physiologically active molecules for breast-fed infants. The investigation of fatty acid (FA) composition, not only in total lipids but also in phospholipids (PLs) contained in human milk, is very important for guidance and adjustment of the diet of lactating mothers, as well as for the improvement of infant formulas. In effect, while PLs can be considered an important source of energy, they also afford long chain polyunsaturated fatty acids (LC-PUFAs), which play a pivotal role in growth and brain development in newborn infants (Wang et al., 2000). In fact, recently it has been reported that the PLs content and fatty acid distribution in PL species should be investigated as functional bioactive elements for the production of functional foods or dietary supplements for the prevention or treatment of specific diseases, and for producing new formulas for infants or adults in the field of artificial nutrition (Benoit et al., 2010; Garcia et al., 2012).

In principle, the composition of human milk has served as a basis for the development of infant formulas. However, the composition of human milk PLs shows variations depending on factors such as lactation time and the nutritional state of the mother, among other aspects. While quantitatively considered to be of only minor relevance, these variations in composition

ultimately may result in differences in biological effects according to whether infants are fed with human milk or infant formulas.

Research has been carried out to establish the organic requirements and consumption recommendations of some milk constituents. Recently, new food ingredients such as choline have been added to infant formulas in Europe, based on scientific evidence (Commission Directive, 2006; EFSA, 2011). However, uncertainties remain with regard to the real needs and levels of some chemical constituents, including PLs. This is related in part to uncertainty as to the nature of the evaluations needed to determine whether the modifications in infant formulas are suitable and safe (Koletzko et al., 2002).

Based on this background, the present review has the following objectives: (i) to offer an update on PL contents and fatty acid distribution in the main PLs found in human milk and infant formulas; (ii) to provide an overview of the main biological effects of PLs related to infant nutrition; and (iii) to comment the limited literature on the PL nutritional needs of newborn infants. As a whole, this review underscores the differences in composition between human milk and infant formulas, and provides a scientific basis for helping to adjust formulations to the infant nutrition requirements.

2. Structure of the main phospholipid classes in human milk

The main compounds of human milk fat are fatty acids, which are esterified mainly in the form of triacylglycerols, accounting for 98% of milk fat. A smaller portion of fatty acids (0.2-2%) is esterified in the form of PLs, which are mainly distributed into 5 major classes: three

predominant (62-80%) (phosphatidylethanolamine (PE), phosphatidylcholine (PC) and sphingomyelin (SM)) and two minor compounds (12-15%) (phosphatidylinositol (PI) and phosphatidylserine (PS)) (Bitman et al., 1984; Braun et al., 2010; García et al., 2012). Although attention is centered on the main classes of PLs present in human milk and infant formulas (PE, PI, PS, PC and SM), other minor classes of PLs with potential health benefits for the newborn infant, such as lysophospholipids and plasmalogens, have also been identified in human milk (García et al., 2012).

Phospholipids and sphingolipids, also known as polar lipids, are amphiphilic molecules with a hydrophobic tail and a hydrophilic head group. The glycerophospholipids (PE, PI, PS and PC) consist of a glycerol backbone on which two fatty acids are esterified in positions *sn-1* (generally more saturated) and *sn-2*. On the third hydroxyl, a phosphate residue with different organic groups (ethanolamine, inositol, serine or choline) may be linked. In contrast, if the backbone of a PL is the long chain amino-alcohol sphingosine (containing 18 carbon atoms, two hydroxyl groups and one double bond) instead of glycerol, sphingophospholipids result - SM being the most representative compound in this case (Rombaut and Dewettinck, 2006; Küllenberg and Taylor, 2012). The structures of the main phospholipids are shown in Figure 1.

PLs in milk are mainly situated in the milk fat globule membrane (MFGM), coexisting with cholesterol, enzymes, glycolipids and glycoproteins (Evers et al., 2008). The distribution of MFGM polar lipids is asymmetrical, with PC and SM largely located in the outer layer of the membrane, while PE, PI and PS are concentrated in the inner surface (Deeth, 1997). This distribution has a great influence upon the microstructure of this highly complex biological membrane, stabilizing the lipidic core of the milk fat globule and protecting it from enzymatic

degradation by lipases (Rombaut and Dewettinck, 2006). In addition, PLs also have a nutritional function as suppliers of long-chain polyunsaturated fatty acids (LC-PUFAs) and choline, which are nutrients needed for optimum development and function in the newborn infant (ESPGHAN, 2005).

3. Phospholipid contents in human milk and infant formulas

3.1 Human milk

Human breast milk is a dynamic system in which fat composition is influenced by factors such as maternal diets, the duration of pregnancy or the stage of lactation. In addition, other potential associated factors comprise the geographical setting, cultural traditions and socioeconomic status (Pita et al., 1985; Smit et al., 2002). There are three phases in breast milk production: colostrum (day 1 to 5 postpartum), transitional milk (day 6 to 15 postpartum), and mature milk (after day 15 postpartum) (Sala-Vila et al., 2005). Traditionally, studies have focused on differences in the total fatty acid composition of human breast and its triacylglycerol composition between different stages of lactation. However, very few studies have addressed the content or relative proportion of the main classes of PLs, and even fewer have examined the influence of the lactation stage upon PL content (Sala-Vila et al., 2005; Zou et al., 2012) or the relative proportions of the main PLs (Harzer et al., 1983; Sala-Vila et al., 2005; Shoji et al., 2006; Zou et al., 2012).

Covering the last 30 years, we have compiled a total of 17 studies providing information on the contents and relative proportions of the main PLs (PE, PI, PS, PC and SM) in human milk

(Table 1). Total PLs in human milk ranged between 9.8-47.4 mg $100g^{-1}$. As a general trend, among the different polar lipid species, SM (28.9-43.3%) and PC (19.0-38.4%) are seen to be the main PLs, followed by PE (5.9-36.1%), PS (3.7-16.7%) and PI (1.1-10.1%), irrespective of the lactation stage. The high content of SM and PC is considered of great importance for the development of infants, since approximately 17% of the total choline (required for rapid organ growth and membrane biosynthesis) received by the neonate comes from these polar lipids (Sala-Vila et al., 2005; Zou et al., 2012).

Harzer et al., in 1983, were the first to study the changing patterns of the distribution of PL species as lactation progresses. These authors found a significant rise in the relative proportions of SM and PE, together with a decrease in PC during the transition from colostrum to mature milk - the concentration of total PLs remaining constant in the course of lactation.

Other studies (Holmes et al., 2000; Sala-Vila et al., 2005; Shoji et al., 2006; Zou et al., 2012) have described some particularities with respect to the initial study of the concentration of PLs during lactation. Holmes et al. (2000) observed no variation in the contents of PC and SM in the transition from colostrum to mature milk. Nevertheless, Sala-Vila et al. (2005) and Zou et al. (2012) reported an increase in PE and PS, no modifications in SM, and a decrease in PC. Shoji et al. (2006) in turn observed a decrease in PC and an increase in SM in human milk from mothers giving birth to pre-term infants (no differences were found on jointly considered the values in term and pre-term groups), with PE, PI and PS remaining unaltered in the course of lactation in both the term and pre-term groups. However, in these studies discrepancies were noted in the variation of total PLs. Sala-Vila et al. (2005) and Shoji et al. (2006) observed a significant

decrease in total PLs, while Zou et al. (2012) reported an increase in total polar lipid content during the course of lactation (reaching a maximum in the transitional stage).

The presence of choline and choline compounds in human milk shows distinct levels, depending on the lactation time. For example, milk produced between 12 and 28 days after birth contains two times more choline and choline compounds ï glycerophosphocholine, PC and free choline being the main representatives (Ilcol et al., 2005).

The mechanism by which SM, PE and PS increase during lactation is unclear, but might involve differences in activity of the mammary gland (Shoji et al., 2006). Whether or not these changes are of nutritional significance to the infant remains uncertain. Nevertheless, in this regard, Tigney (1956) showed the SM and PE content of the human brain to increase significantly during the first year of life. Consequently, the requirements of these PLs increase and must be covered by the diet of the neonate. In this context, the tendency towards increased SM, PE and even PS in prolonged lactation can represent a compensatory mechanism, acting as a choline supplier for ensuring optimal development and function of the newborn infant.

In order to conduct a review based on the best available evidence, we classified the different studies relating PL contents and/or relative proportions in human milk (Table 1) according to different parameters: the number of samples used in the study, representation of sample size, mode of human milk extraction, extraction period of milk during sampling, sample preservation, methodology and method validation (Table 2). In addition, we classified the studies from suitable to highly suitable, based on three main aspects: representation of sample size (if ≥ 20 samples scoring ++, if not = none), extraction period (if using all nursing-day times, mix of nursing-day times or foremilk scoring +, if not = none) and methodology validation (precision,

accuracy, limit of detection (LOD), limit of quantitation (LOQ), and linearity) (if yes scoring +, if not = none).

Apart from ethical reasons and the motivation of mothers for enrollment in clinical studies, with willingness to supply their milk, studies must be adequately powdered through a representative size of the population. In this context, of the 17 studies reviewed, 12 showed the best contemplated score regarding sample size (≥ 20 samples).

Regarding the extraction period, it has been reported that the use of milk samples at the beginning of milking (foremilk) minimizes all factors that might influence milk composition, apart from the stage of lactation, because milk obtained after complete expression of the breast (hindmilk) has a significantly higher content of lipids and PLs (Zeisel et al., 1986; Sala-Vila et al., 2005; Shoji et al., 2006). The use of all nursing-day times or mix of entire feed also avoids the problems of modifications that may occur during the course of feeding from foremilk to hindmilk (Holmes et al., 2000). Eight of the 17 studies reviewed adhered to this guideline. Preservation of samples is also important, and it has been reported that freezing at -80ºC inactivates human milk lipases, as a result of which the PL contents remain the same as in the fresh milk (Zeisel et al., 1986). On the other hand, although mechanical expression of human milk would be expected to yield more homogeneous samples than milk from manual breast emptying, no evidence of differences has been found in the reviewed studies.

Another aspect to bear in mind is the methodology used to analyze PLs in human milk. The techniques range from more traditional thin layer chromatography (TLC) to more sophisticated techniques such as mass spectrometry (MS). TLC is mainly used for qualitative and semi-quantitative purposes. This technique is flexible, easy to use and relatively inexpensive.

However, for quantification purposes, TLC is time consuming and should be scrupulously validated and standardized, because staining intensity is time- and matrix-dependent (Rombaut and Dewettinck, 2006). Nuclear magnetic resonance (^{31}P NMR) does not need prior solvent extraction typical of TLC and high-performance liquid chromatography (HPLC). In contrast, this technique requires important investment in high resolution equipment and skilled operators (Rombaut and Dewettinck, 2006; Fong et al., 2013).

By far the most commonly reported analytical method for PL quantification is HPLC coupled to either MS or an evaporative light-scattering detector (ELSD) for detection. ELSD is compatible with a broad range of solvents and gradient elution, and the signal is independent of the degree of saturation and length of an acyl chain (unlike the UV detector). Nevertheless, this detector is limited by its low identification potential, low selectivity and nonlinearity, giving rise to complicated calibrations with linearity over only small concentration ranges (Restuccia et al., 2012; Fong et al., 2013). The advantage of MS detection of PLs is that it offers greater specificity and selectivity, and in many cases greater sensitivity than UV, ELSD or ^{31}P NMR (Fong et al., 2013). However, it is expensive, and both UV and MS detectors can suffer from non-uniform responses due to differences in absorptivity and ionization efficiencies as a function of chemical structure (Restuccia et al., 2012). Each methodology has its advantages and inconveniences; however, what is of relevance is the fact that the method has been validated (in terms of precision, accuracy, LOD, LOQ and linearity) to obtain comparable and reproducible results. Four of the reviewed studies had method validation and four reported partial validations, while 9 performed no validations.

In general, we found the results to be comparable, despite great discrepancies among the studies in terms of: (i) the number of samples analyzed and representation of the sample size; (ii) the kind of results presented (total and/or individual PLs, contents and/or relative proportions, one or all stages of lactation, and milk extraction period (foremilk, middle milk and/or hind milk) during sampling); and (iii) the methodology used (TLC, gas chromatography (GC), HPLC and NMR). Nevertheless, considerations referred to sample size, extraction period and method validation should be taken into account in order to use more rigorous methods and criteria in the design of studies of phospholipids in human milk.

3.2 Infant formulas

The contents of the major PL subclasses in different infant formulas found in the literature in recent years are shown in Table 3. PL contents (mg $100g^{-1}$) in each polar lipid reported ranged from 1.5-75.0 (PE), 0.6-46.0 (PI), 0.6-28.0 (PS), 1.-84.0 (PC) and < 0.4-82.0 (SM). As a particularity, referred to the study published by Fong et al. (2013), Table 3 does not include the values of an infant formula with colostrum powder added, since it presented far higher contents of PE (143 mg $100g^{-1}$), PI (60 mg $100g^{-1}$), PC (253 mg $100g^{-1}$) and SM (125 mg $100g^{-1}$) than all the other infant formulas analyzed in the studies made with whey-protein-dominant powder.

Globally considering the intervals of the 8 studies on PL contents in infant formulas (Table 3), it seems that the polar lipid contents fall within the range observed in human milk, or are even higher (see Table 1). However, differences in PL contents in human milk versus infant formulas are found when individual studies are considered (Zeisel et al., 1986; Kynast et al.,

1988; Holmes-McNary et al., 1996; Holmes et al., 2000; Ilcol et al., 2005). In addition, PL contents vary greatly among the different groups of infant formulas investigated (i.e., adapted, partially adapted, special formulas, bovine-derived formulas and soy-derived formulas), and also within the same groups of infant formulas - thus indicating that manufacturers use different ingredients for the elaboration of these products (Kynast et al., 1988).

In this context, Zeisel et al. (1986) reported that infant formulas made from bovine milk had similar amounts of PC but less SM than bovine milk. However, soy-derived formulas contained more PC and again much less SM than bovine or mature human milk. Similarly, Holmes-McNary et al. (1996) indicated that human milk PC and SM were not different from bovine-derived formulas, but in the case of soy-derived formulas more PC and less SM was detected. As a general trend, infant formulas contain less SM than human milk - a fact also confirmed in other studies (Holmes et al., 2000; Ilcol et al., 2005). However, in the case of PC, contradictory results are found, since the abovementioned studies (Zeisel et al., 1986; Holmes-McNary et al., 1996) reported similar or higher PC contents in infant formulas versus human milk, while the opposite has been reported by other authors (Holmes et al., 2000; Ilcol et al., 2005).

Another aspect to bear in mind is that in general, the PC content in infant formulas is higher than the SM content. This contrasts with the relative proportion of these two PLs found in human milk, where SM is usually present in higher proportions than PC. This can be attributed to the fact that infant formulas are generally instantized using soya lecithin (PC) (Fong et al., 2013).

Consumption of breast milks and infant formulas with different choline contents and compositions can directly affect serum free choline levels in breast-fed and formula-fed infants,

and can have an effect upon brain development (Ilcol et al., 2005). Indeed, clinical studies show that breast-feeding is associated with significantly higher scores for cognitive development than formula feeding (Anderson et al., 1999). Because there are critical demands for choline in neonates, it is very important to ensure adequate availability of the compound through proper infant nutrition (Holmes-McNary et al., 1996). To achieve this goal by means of infant formulas when breast-feeding is not possible, choline suppliers such as PC and SM should be included in a quantity and proportion that best emulate the composition of human milk, in order to cover perinatal choline requirements.

4. Fatty acid composition of phospholipids in human milk

The fatty acid composition of human milk has been extensively analyzed in several studies. Nevertheless, the esterification of fatty acids into PLs (total or individual), and the influence of the stage of lactation, have been less widely addressed in the literature (Morrison et al., 1967; Harzer et al., 1983; Bitman et al., 1984; Wang et al., 2000; Sala-Vila et al., 2005; Benoit et al., 2010; Blaas et al., 2011; Zou et al., 2012). This is of relevance, since it has been reported that the fatty acid compositions of human milk fat and of PLs have different origins, probably due to differences in diet, genetics and environmental factors, and this difference might be of biological importance for the development of infants (Zou et al., 2012). In addition, PLs can be considered an important source of energy, as well as providers of LC-PUFAs, which play a pivotal role in the growth and brain development of neonates (Wang et al. 2000).

The fatty acid composition of individual PLs in human milk as reported in 5 studies (Morrison et al., 1967; Bitman et al., 1984; Wang et al., 2000; Benoit et al., 2010; Blaas et al., 2011) is summarized in Table 4. Major compounds (each representing approximately Ó10%) or fatty acids of high nutritional relevance involved in child growth, visual acuity and neurological development (Campoy et al. 2012), such as arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic (DHA), appear in boldface.

In general, PC, PI and PS exhibit a similar distribution (~ 50%) of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). In the case of PE there are more UFAs (~ 65%), while SM presents a high distribution of SFAs (70-80%). Globally, palmitic acid (C16:0) was the main fatty acid in PC, stearic acid (C18:0) in PE, PI and PS, and behenic acid (C22:0) in SM. Regarding fatty acids of nutritional relevance, AA (C20:4), EPA (C20:5) and DHA (C22:6) are mainly present in PE, PI and PS.

Changes in the fatty acid composition of milk PLs occur throughout the course of lactation (during the first 2-3 weeks), probably implying a shift in the infant from brain cell division to myelinization (Crawford et al., 1981; Bitman et al., 1984). Some authors have indicated that the fatty acid composition of PLs from different lactation stages shows that no remarkable or regular changes can be generalized (Zou et al., 2012). However, while this latter assumption is true, modifications in fatty acids in total PLs and in the different polar lipid subclasses have been described and may have distinct nutritional and biological consequences. Thus, regarding fatty acids in total PLs, a decrease can be observed in C16:0, with an increase in C18:0, C18:2 and EPA. C18:1 does not change during lactation, and AA and DHA do not show a regular trend (Harzer et al., 1983; Sala-Vila et al., 2005; Zou et al., 2012).

Regarding fatty acids in individual PLs, the high SFA content of SM, and the fact that two-thirds of the fatty acids are long-chain fatty acids (LC-FAs) (with C22:0, C24:0 and C18:0 as the major compounds), represent two physical properties which contribute to lessen fluidity and tend to support a structural role for SM in maintaining rigidity of the MFGM. The decreases that occur during early lactation in C16:0, and the increases in C22:0, C24:0 and C24:1, are consistent with this structural role of SM (Bitman et al., 1984; Zou et al., 2012). On the other hand, glycerophospholipids (PE, PI, PS and PC) suffer an increase in their unsaturated nature from 50% to 60% during the course of lactation - the degree of unsaturation increasing in the order PE > PI > PS > PC. In this sense, all glycerophospholipids show an increase in C18:2 from colostrum to mature milk (Bitman et al., 1984). This fact is important, since this essential fatty acid is a precursor (through desaturation and chain elongation) of AA, which plays a major structural role in biological membranes and functions as a precursor of eicosanoids of the *n-6* series (Benoit et al., 2010). In fact, AA also exhibits an increase during the course of lactation from about 2% to 8%, in the order PE > PI > PS > PC (Bitman et al., 1984).

Other fatty acids of nutritional relevance, such as EPA and DHA (involved in the development of visual acuity and the nervous system (Campoy et al., 2012)) have been found in much higher quantities in PE and PI than in PC, and may be regarded as a source of these LC-PUFAs for infants in the rapid developmental stage (Wang et al., 2000), since they also show an increase in the course of lactation (Bitman et al., 1984).

To our knowledge, no study has addressed the fatty acid composition of PLs in infant formulas. Only recently, Fong et al. (2013) have tentatively identified some PC molecular ions by HPLC-MS/MS in infant formulas as fatty acid combinations C16:0/C18:2 and C18:2/C18:2

that are commonly found in soya lecithin. These authors open a window for using this sensitive and specific technique to explore the fatty acid composition of PLs. On the other hand, on considering ingredients used for infant formula preparation, such as bovine MFGM, some differences are observed versus human milk. In this sense, as reported by Benoit et al. (2010), all PL species (PC, PE, SM and PI+PS) in cow MFGM showed more C18:1 than human milk. In contrast, lesser proportions of C18:0 (in PC, PE and SM), C18:2 (in PC), AA (in PC, PE, and PI+PS), EPA (in PI+PS) and DHA (in PE and PI+PS) were found in cow MFGM versus human milk. Therefore, infant formulas should mirror the fatty acid content in healthy breast milk, referring not only to their contents in total lipids, but also in terms of PLs.

5. Biological effects of phospholipids

Forepart from their importance in emulsifying the globules of fat in milk, PLs from the fat fraction of human milk have interesting properties associated with biological function. For newborn infants and small children, PC and SM are the main sources of choline (Zeisel, 2006), and jointly account for about 40-50% of cellular membrane composition (Emmelot and Van Hoeven, 1975, Zeisel, 2006). Choline is a precursory amino alcohol of the neurotransmitter acetylcholine. It acts by regulating the transduction signal, and serves as a source of methyl groups in intermediate metabolism, being considered essential for optimum development of the brain (Zeisel and Blusztajn, 1994; Zeisel, 2006). Accordingly, energetic ingestion and the chemical composition of the diet are among the important factors influencing the functions of the brain. A study carried out in male mice of three months of age administered a diet with a low

energetic value during two months reported higher PC and SM contents in the hippocampus and neocortex, respectively. On the other hand, the level of PE in these structures was found to be reduced when compared with the animals receiving a control diet. The rise in the amount of PC in the brain as a result of the diet of low energetic value was significantly correlated to improvement in conditional reflex activity (Babenko and Shakhova, 2012).

In most eukaryotic cells, PC and PE are synthesized by an aminoalcoholphosphotransferase reaction using a biosynthetic pathway known as the Kennedy pathway, thus named after Eugene Kennedy, who elucidated it over 50 years ago (Gibellini and Smith, 2010). Using an animal model of both genders, it was seen that PC metabolism could vary according to gender and the diet consumed. The liver of males was found to be more dependent upon the Kennedy pathway for PC synthesis than the liver of female mice. The latter produce PC via a pathway that uses phosphatidylethanol-amine *N*-methyltransferase (PEMT) to convert PE to PC, while male mice make lesser use of this pathway to produce PC (Noga and Vance, 2003). The biological actions of PC include promotion of the synthesis of important neurotransmitters for memory and brain development (Caudill, 2010; Li and Vance, 2008).

Apart from being source of choline, PC allows the liver to recover from toxic or chronic viral damage through the donation of methyl groups for hepatic regeneration. A protective effect of PC against pharmaceutical and death cap mushroom poisoning, alcoholic liver damage and the hepatitis B virus has been cited in the literature (Kidd, 2002). A study in male mice aged 4-5 weeks and fed for three weeks with a lipid-rich diet supplemented with PC from soy or egg (hydrogenated and non-hydrogenated) has recently been published. The results evidence that the liver lipids decrease after PC supplementation (hydrogenated or non-hydrogenated PC), and this

reduction is independent of the dietetic content of polyunsaturated fatty acids - thus supporting the hypothesis that the ability of dietetic PC to lower liver lipid levels is not due to its fatty acid content (Tandy et al., 2010).

The SM molecule and its metabolites have important actions in many types of cells, and also act upon intracellular messengers involved in regulatory processes and in inductive cell apoptosis (Ribar et al., 2007). The digestion of dietary SM is catalyzed by the intestinal enzyme alkaline sphingomyelinase (SMase), and has been shown to be incomplete. Accordingly, enterocytes can be exposed to certain amounts of indigestible SM and its degradation products, including ceramide (Duan and Nilsson, 2009; Liu et al., 2000).

The partial digestion of SM in the small intestine is related to the presence and interference of other compounds such as bile salts, glycerophospholipids and their hydrolytic products that inhibit the activity of SMase. Additionally, pH 7.4 (similar to the pH found in the small intestine) and the presence of certain PLs, such as PC, PS, PI and PE, are also capable of inhibiting the activity of SMase in a dose-dependent manner (Liu et al., 2000). The presence of intact SM and its metabolites has important implications in the development of colon tumors (Liu et al., 2000; Duan and Nilsson, 2009), and also in inflammatory processes, cell functions and growth (Duan and Nilsson, 2009).

Experiments in female mice (5 weeks of age and virus antibody-free) administered 0.1% (w/w) of SM purified from bovine milk in the diet showed reductions of close to 70% in the number of aberrant colonic crypt foci and of about 30% in the number of aberrant crypts per focus ï both of these parameters being considered initial markers of colon carcinogenesis. The consumption of SM did not affect the incidence or the multiplicity of colon neoplastic disease,

though the animals that had received SM presented adenomas, while those administered a diet without SM developed adenocarcinoma ï with no differences in body weight gain (Schmelz et al., 1996). Other investigators have also obtained results that evidence the benefit of SM in the initial stage of colon cancer in experimental studies in animals and cell cultures (Vesper et al., 1999; Schmelz et al., 2000; Schmelz et al., 2001).

In addition to the chemoprotective and chemotherapeutical properties of SM from bovine milk (Lemonnier et al., 2003), this sphingolipid has other benefits for human health. Such benefits include hypocholesterolemic action, as reported in some studies (Vesper et al., 1999; Rombaut and Dewettinck, 2006). SM can reduce cholesterol absorption between 20.4-85.5%, depending on the ingested dose (0.1% and 5.0%, respectively) (Rombaut and Dewettinck, 2006). These concentrations include the estimated sphingolipid consumption level among North Americans (0.025-0.5% of the diet) (Vesper et al., 1999). It is important to mention that SM in bovine milk inhibits cholesterol absorption more intensely than in other alimentary sources (Rombaut and Dewettinck, 2006).

However, on establishing comparisons between human milk and infant formulas, it has been shown that the latter are produced aiming at obtaining a product closer to human milk, but they contain significantly lesser amounts of sphingolipids, as previously indicated in section 3.2 of this review. Investigators suggest that this difference, as well as the existing distinct lipid profile between human and bovine milk, could affect growth, brain development, the risk of food allergies, and non-immunoglobulin actions against bacterial toxins (Ribar et al., 2007; Vickers et al., 2009).

Nutritional supplementation with a complex milk lipid (CML) preparation with 46% (w/w) of PLs in the lipid fraction, carried out in Wistar rats with an age of 100 days, resulted in significantly increased growth rates, with no changes in body composition. The animals administered CML exhibited better learning capacity (recognition and space memory), suggesting that supplementation had a positive effect upon learning behavior and normal growth (Vickers et al., 2009). Gustavsson et al. (2010) have also reported differences in learning behavior and postnatal growth among male animals born from rats that had received supplementation with a CML containing gangliosides and PLs during pregnancy and lactation. While growth and food intake were not modified, the offspring of the supplemented rats presented greater brain weight, with higher ganglioside levels and lower levels of PLs. On reaching adulthood, these animals had less body fat, but without differences in learning and recognition capacity. The authors suggested that it would be safe to supplement pregnant and breast-feeding women with CLM, resulting in a possible positive impact upon brain weight and ganglioside and PL contents.

Regarding PS, some health benefits have been attributed to this compound from bovine milk, including the capacity to attenuate neuronal age-related effects and memory normalization for the conduction of a series of tasks. However, such benefits has been associated with high supplementation levels (200 mg/day) that are in contrast to the small amounts found in milk and milky products (Pepeu et al., 1996).

In neonatal rats, brain development was examined in response to the use of the same levels of polyunsaturated fatty acids (C18:3 n-3, C20:4 n-6 or C22:6 n-3) proposed in infant formulas. Nursing dams at parturition and subsequently weaned pups were fed diets varying in n-

6 to n-3 fatty acid ratio, with or without C20:4 n-6 and C22:6 n-3 alone or in combination, until 6 weeks of age. The results revealed that the dietary changes in fatty acid profile significantly affected the composition of the neuronal and glial cell membranes in both genders. The PI and PS composition was distinct and showed modifications with age. Alterations in brain fatty acid composition reflected the fatty acid composition of the diet provided, and if the results can be extrapolated, feeding children with C20:4 n-6 and C22:6 n-3 or a reduced C18:2 n-6 to C18:3 n-3 ratio could modify the fatty acid profiles of human brain cells (Jumpsen et al., 1997).

However, not all human cells are influenced by the fatty acid composition of the diet. A study in young male rats demonstrated that the PL composition of the ocular lens is strictly regulated and seems to be independent of the lipids consumed in the diet (Nealon et al., 2008). Apart from the composition of the diet, exercise has been shown to influence and modify the PL profile of cell membranes. The PI content was found to decrease between 36-57% among rats receiving exercise and a controlled diet, with increased levels of those fatty acids that are found in PC and PE. It has been concluded that exercise with a controlled diet, but not exercise alone, significantly reduce body weight and fat, and modify the PL profile (Ouyang et al., 2010).

6. Requirements and nutritional recommendations for phospholipids

The recommended choline intake in the postnatal period is about 125, 150 and 200 mg/day at 0-6 months, 6-12 months and 1-3 years, respectively (IOM, 1998). However, these values are not reached in preterm infants, as has recently been shown by Bernhard et al. (2012). In their study, adequate intake of choline in preterm infants was estimated from the international

recommendations for infants, children and adults, based on the calculations of human milk content for 0-6 month-old infants. Choline intake was determined retrospectively in infants with extremely low birth weight (below 1000 kg) or with a gestational age of under 28 weeks. A total of 93 infants participated in the study, with data collection between day 0 and day 98 (d0-d98). Results showed that children with 0.290 kg body weight needed more choline than those weighing 1200 kg (31.4 and 25.2 mg/kg/day, respectively). The median choline supply reached a plateau at d11 (21.7 mg/kg/day; 25th/75th percentile: 19.6/23.9). Individual choline supply at d0-d1 and d2-d3 was < 10 mg/kg/day in 100% and 69% of the infants, respectively. The study showed that intakes of < 10 mg/kg/day were frequently observed beyond day 11, with median adequate intakes (27.4 mg/kg/day at 735 g body weight) in only < 2% of the cases.

The choline content in human milk can influence circulating choline status in infants and, the choline contents in human milk vary between women and milk free choline contents are influenced by women circulating choline status (Ilcol et al., 2005). A study in breastfeeding women indicated that the PC values in milk and in plasma are correlated to the dietetic intake of choline. Those women who had received choline supplements subsequently presented increases in choline, betaine and PC in milk, and increases in choline and betaine in plasma. The study also analyzed the effects of genetic polymorphisms upon the metabolism of choline. It was concluded that the dietetic ingestion of choline and also the genotype influence the concentration of choline and its metabolites in the milk and plasma of breastfeeding women, thereby affecting the available amount of choline for the infant (Fischer et al., 2010).

In rat milk, a study has demonstrated that the concentration of choline reflects the dietetic consumption of the animals. Gestating mice receiving a diet deficient in choline presented a 50%

decrease in PC, without modifications of the others compounds containing choline. On the other hand, when female mice consumed a diet supplemented with choline, the concentration of PC in milk increased (Holmes-McNary et al., 1996). Also in mice, the intraperitoneal administration of choline chloride (4-60 mg/kg) has been shown to increase choline metabolites in rat brain and plasma, particularly as regards PC ï this indicating delayed transfer of newly taken-up choline into membrane choline pools (Klein et al., 1992).

Researchers had reported that there are significant differences in bioavailability, tissue uptake and metabolism among the choline compounds that are present in rat milk, being proposed that choline and PC appear to be essentially similar in their absorption and metabolic fate (Cheng et al., 1996). However, questions relating to the bioavailability of choline in infant formulas remain to be clarified (Zeisel, 2006).

In humans, studies have been made of the variations in fatty acid contents of the diet and their effects upon the composition of PC and PE in the erythrocytes of children. The erythrocyte membrane of the children between 4.5 and 6 months age administered human milk presented PC and PE with significantly more 20C and 22C polyunsaturated fatty acids than those who received commercial infant formulas (Putman et al., 1982).

In the year 2005, the Committee on Nutrition of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposed a global standard for the composition of infant formulas. The standard includes 7 and 50 mg of choline/100 kcal as the recommended minimum and maximum values, respectively (ESPGHAN, 2005). More recently, the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies issued an opinion related to choline. The Panel considers that choline is sufficiently characterized

and can be used in health claims. The health benefits related to choline are: contribution to normal lipid metabolism, maintenance of normal liver function, contribution to normal homocysteine metabolism, maintenance of normal neurological function, contribution to normal cognitive function, and brain and neurological development (EFSA, 2011).

In relation to SM, a compound with a high degree of biological activity (Rombaut and Dewettinck, 2006), there is little evidence that the dietetic consumption of SM is necessary for growth under normal conditions once the body proves able to synthesize the molecule. The estimated daily dietetic intake among North American adults is between 0.3-0.4 g/day (Vesper et al., 1999). As previously stated in section 3.1, SM comprises 28.9-43.3% of the total human milk PLs, and represents the most prevalent form. For a preterm infant, it has been estimated that an intake of 170 ml of human milk provides 13 mg of SM (Garcia et al., 2012).

However, there are few data on the nutritional importance of SM for neonates and infants ï this compound potentially being involved in the growth and development of tissues through the regulation of cell proliferation and differentiation. The changes in the gastrointestinal tract in the postnatal period were evaluated in rats that received artificial formula based on cowôs milk. The small intestine of these animals was approximately 20% longer and 60% greater in wet weight than in the case of rats fed with rat milk or a control diet. Morphologically, the rats that receive the milk substitute demonstrated significantly greater intestinal villus length and crypt depth compared with the others rats groups. The activity of the intestinal enzyme sucrase was significantly elevated in the group of rats fed milk substitute, compared with rats receiving milk or control diet. The authors concluded that the artificial formula is a principal cause of small intestine overgrowth and precocious maturation of some intestinal functions (Dvorak et al.,

2000).

In another study in rats receiving milk containing 0.5% SM or 0.5% PC during one week, SM in milk was found to play an important role in neonatal gut maturation during the suckling period. Histological examination showed the intestinal villi in the control group and PC group to contain vacuolated cells, whereas in the SM group vacuolated cells were restricted to the tip of the villi. The authors estimated that SM consumption among breast-fed infants should range from 50-150 mg/day in order to promote gut maturation and development (Motouri et al., 2003).

Recently, Garcia et al. (2012), who identified and quantified PLs in milk from different species, calculated that an infant born to term that receives 800 ml of human milk effectively consumes about 62 mg of SM daily. In turn, in the case of a pre-term infant, an intake of 170 ml of human milk provides 13 mg of SM daily. In order to reach the same daily amount of SM with cowôs milk, the authors estimated that 3480 ml and 739 ml of cowôs milk would be required for term and pre-term infants, respectively.

Despite knowledge of the structure and presence of others PLs such as PE and PS in human milk, there is a lack of studies on their functions and requirements in newborn infants and older children. This in turn precludes the definition of nutritional recommendations for their presence in infant formulas. The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies has issued an opinion related to PLs. These compounds are considered to lack sufficient characterization to warrant their claims. The Panel position on PLs is negative in reference to the protection of DNA, proteins and lipids from oxidative damage, memory, learning capacity and attention, and function of the nervous system - the proposed

source of PLs being an animal lecithin-water emulsion derived from the brain tissue of domestic pigs (EFSA, 2009).

7. Conclusions

Despite the many efforts to produce infant formulas as similar as possible to human milk, there are differences referred to PL content and PL fatty acid distribution. These differences can imply biologically significant differences for newborn infants fed formulas versus human milk, mainly as a consequence of the higher proportion of SM versus PC in the former. In addition, the lack of studies characterizing fatty acid distribution among PL species in infant formulas or ingredients used to enrich these products points to the need for further research in order to obtain products with benefits similar to those of human milk in terms of child growth, visual acuity and neurological development. In addition, there is a lack of nutritional recommendations referred to PLs in the field of infant nutrition. Thus, there is a need to establish these values, and further studies on this subject are warranted in order to determine the effective PL doses affording optimal growth and health benefits for infants.

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Table 1. Contents and relative class proportions of phospholipids in human milk in different stages of lactation.

Reference	Lactation stage	Phospholipids (mg 100 g^{-1})						Relative proportion of phospholipids (%)				
		PE	PI	PS	PC	SM	Total	PE	PI	PS	PC	SM
Harzer et al. 1983	*Colostrum*						39.3 ± 0.6	25.1 ± 2.5	5.5 ± 0.9	10.2 ± 2.4	31.9 ± 3.0	28.9 ± 0.6
	Transitional						40.5 ± 2.1	28.0 ± 0.1	5.2 ± 0.1	8.9 ± 0.8	28.2 ± 1.1	30.4 ± 0.7
	Mature						39.0 ± 1.0	27.6 ± 1.0	5.3 ± 0.2	9.1 ± 0.6	25.6 ± 1.0	32.1 ± 0.9
Bitman et al. 1984	*Colostrum*											
	Transitional											
	Mature						15.0-20.0	19.7 ± 0.2	6.0 ± 0.6	8.6 ± 0.2	27.8 ± 1.5	37.8 ± 1.5
Zeisel et al. 1986[*]	*Colostrum*											
	Transitional											
	Mature				11.2 ± 2.5	13.5 ± 1.8						
	Colostru											

	m									
Hundrieser et al. 1988	*Transitional*									
	Mature					23.8 ± 3.3	5.3 ± 3.0	3.7 ± 1.5	33.2 ± 5.5	29.0 ± 6.4
	Colostrum									
Kynast et al. 1988	*Transitional*									
	Mature	7.0-10.0	0.5-2.0	2.0-7.0	8.0-12.0					
	Colostrum									
Holmes-McNary et al. 1996*	*Transitional*									
	Mature			6.6 ± 0.4	8.6 ± 1.1					
	Colostrum									
Wang et al. 2000	*Transitional*					36.1 ± 3.9	3.5 ± 1.5	6.7 ± 4.1	23.1 ± 4.2	30.6 ± 6.6
	Mature									
	Colostrum			17.7 ± 5.4	6.8 ± 0.8					
Holmes et al. 2000*	*Transitional*									

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	Mature	37.0 ± 5.4	7.5 ± 0.8

Results are presented as mean ± SD, or interval (minimum-maximum) depending on the cited reference. * Conversion factors of 751 and 770 g mol^{-1} were used for SM and PC, respectively. PE: phosphatidylethanolamine. PI: phosphatidylinositol. PS: phosphatidylserine. PC: phosphatidylcholine. SM: sphingomyelin.

Table 1. (Continued).

Reference	Lactation stage	Phospholipids (mg 100 g^{-1})						Relative proportion of phospholipids (%)					
		PE	**PI**	**PS**	**PC**	**SM**	**Total**	**PE**	**PI**	**PS**	**PC**	**SM**
Ilcol et al. 2005*	*Colostrum*				11.2 ± 1.4	9.7 ± 1.0							
	Transitional												
	Mature				8.0 ± 0.8	7.1 ± 0.7							
Sala-Vila et al. 2005*	*Colostrum*						13.5 ± 2.6	5.9 ± 0.6	6.0 ± 0.6	7.9 ± 1.1	38.4 ± 3.1	40.5 ± 3.6
	Transitional						14.0 ± 2.5	8.6 ± 1.2	5.2 ± 0.5	8.2 ± 1.0	37.7 ± 4.9	39.2 ± 3.6
	Mature						9.8 ± 1.5	12.8 ± 1.2	5.9 ± 0.5	10.4 ± 1.3	31.3 ± 4.8	41.0 ± 3.4
Shoji et al. 2006	*Colostrum*							14.5 ± 0.7	7.3 ± 1.1	5.3 ± 1.1	28.6 ± 7.8	36.0 ± 0.1

	Transitional							15.0 ± 1.4	7.3 ± 0.4	5.3 ± 0.4	25.5 ± 0.7	37.5 ± 2.1
	Mature							13.5 ± 0.7	7.0 ± 1.4	6.0 ± 0.1	24.9 ± 3.3	39.2 ± 4.5
Benoit et al. 2010	*Colostrum*											
	Transitional											
	Mature							21.3 ± 4.7			19.0 ± 2.2	43.3 ± 2.6
Fischer et al. 2010[*]	*Colostrum*											
	Transitional											
	Mature				8.2 ± 0.5	5.0 ± 0.3						
Blaas et al. 2011	*Colostrum*											
	Transitional											
	Mature					3.1-9.1						
Lopez et al. 2011[+]	*Colostrum*											
	Transitional											
	Mature	1.8 ± 0.6	1.4 ± 0.1	2.1 ± 0.6	2.9 ± 0.6	5.3 ± 0.3	13.5 ± 2.0	14.3 ± 2.7	10.1 ± 1.4	15.4 ± 3.1	21.1 ± 2.2	40.2 ± 4.7
	Colostrum	1.6 ± 0.1	1.4 ± 0.1	2.1 ± 0.1	4.8 ± 0.7	6.9 ± 1.0	16.8 ± 1.5	9.3 ± 1.1	8.3 ± 1.0	12.7 ± 1.6	28.5 ± 1.6	41.2 ± 2.0

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Zou et al. 2012[+]	*Transitional*	2.9 ± 0.5	1.5 ± 0.1	3.1 ± 0.2	5.7 ± 0.5	9.0 ± 1.5	22.3 ± 1.3	13.2 ± 0.9	6.8 ± 0.4	13.9 ± 1.6	25.7 ± 2.2	40.4 ± 3.3
	Mature	2.9 ± 0.4	1.6 ± 0.1	3.2 ± 0.2	4.1 ± 0.4	7.5 ± 1.3	19.2 ± 1.4	15.0 ± 1.5	8.2 ± 1.2	16.7 ± 1.8	21.3 ± 2.4	38.8 ± 3.9
Garcia et al. 2012	*Colostrum*											
	Transitional											
	Mature	2.6-10.3	0.2-2.1	1.1-4.5	3.2-12.4	5.0-13.3	15.3-47.4	12.4-25.6	1.1-5.2	5.4-15.2	19.8-30.2	25.7-33.8

Results are presented as mean ± SD, or interval (minimum-maximum) depending on the cited reference. [*] Conversion factors of 751 and 770 g mol^{-1} were used for SM and PC, respectively. [+]mg 100 g^{-1} calculated considering that human milk contains 3.8 g of fat per 100 g (Ballabriga and Carrascosa, 2006). PE: phosphatidylethanolamine. PI: phosphatidylinositol. PS: phosphatidylserine. PC: phosphatidylcholine. SM: sphingomyelin.

Table 2. Parameters reported in human milk phospholipid studies used to assess the suitability of the results obtained.

Reference	Nº samples	Representation[1]	Extraction	Extraction period[2]	Sample preservation	Method	Method validation
Harzer et al. 1983	308	+	Mechanical	All nursing-day times	- 30 ºC	TLC-densitometry and GC-FID	No
Bitman et al. 1984	123	+	Mechanical	Hindmilk	- 20 ºC	TLC-densitometry and GC-FID	Accuracy (recovery assays)
Zeisel et al. 1986	292	+	Manual/Mechanical	All nursing-day times	- 20 ºC	TLC-densitometry	Yes
Hundrieser et al. 1988	8	-	Mechanical	Middle milk	- 70 ºC	HPLC-UV	Yes
Kynast et al. 1988	2	-	Manual	Hindmilk	4 ºC	HPLC-UV	No
Holmes-McNary et al. 1996	33	+	Mechanical	Hindmilk	- 80 ºC	HPLC-radiometry and GC-MS	Accuracy (recovery assays)
Wang et al. 2000	20	+	Manual	Unknown	- 80 ºC	TLC-densitometry and GC-FID	No
Holmes et al. 2000	8	-	Manual	Mix of nursing-day times	- 20 ºC	^{1}H NMR	Accuracy (recovery assays)
Ilcol et al. 2005*	116	+	Manual	Foremilk	Ice and analysis	TLC-densitometry and colorimetric method	No
Sala-Vila et al. 2005*	66	+	Mechanical	Foremilk	- 80 ºC	HPLC-ELSD	Yes

Shoji et al. 2006	34	+	Unknown	Foremilk	- 80 ºC	TLC and phosphorus analysis	No
Benoit et al. 2010	4	-	Mechanical	Mix of nursing-day times	- 80 ºC	TLC and GC-FID	No
Fischer et al. 2010*	103	+	Mechanical	Hindmilk	- 80 ºC	HPLC-MS/MS	Yes
Blaas et al. 2011	20	+	Unknown	Unknown	- 18 ºC	HPLC-MS/MS	Accuracy (recovery assays)-linearity
Lopez et al. 2011+	3	-	Mechanical	Foremilk	4 ºC	HPLC-ELSD	No
Zou et al. 2012+	45	+	Unknown	Unknown	- 20 ºC	HPLC-ELSD	Accuracy (recovery assays)
Garcia et al. 2012	22	+	Unknown	Hindmilk	Frozen	^{31}P NMR	No

[1](+) if ≥ 20 samples. (-) if < 20 samples. [2]Nursing times: prior (foremilk), after (middle milk) and after complete breast emptying (hindmilk). [3] +, ++, +++, ++++ = suitable to highly suitable, according to representation, extraction period and method validation.

Table 3. Phospholipid contents in different infant formulas.

Reference	Infant formulas	Phospholipids (mg 100 g^{-1})					Method
		PE	PI	PS	PC	SM	
Zeisel et al. 1986*	Bovine milk based				1.8-14.4	n.d.-0.5	TLC-densitometry
	Soya based				18.2-19.1	n.d.	
Kynast and Schmitz, 1988	Adapted	3.4-7.0	1.5-5.0		3.7-11.7	6.7-7.4	HPLC-UV
	Partially adapted	1.5-2.5	0.6-1.0		1.8-4.4	1.6-2.7	
	Special	n.s.-15.7	n.s.-32.4		n.s.-62.3	n.s.-13.0	
Holmes-McNary et al., 1996	Bovine milk based				2.2-7.7	0.8-4.3	HPLC-radiometry andGC-MS
	Soya based				10.0-16.6	0.8-2.9	
Holmes et al. 2000	Adapted				6.9-9.2	2.3-3.8	^{1}H NMR
	Special				3.1-7.7	2.3-7.5	
Sala-Vila et al. 2003	Bovine milk based	2.2	1.4	0.6	6.2	1.0	HPLC-ELSD
Ilcol et al. 2005	Bovine				3.8-9.9	0.4-1.7	TLC-

	milk based						densitometry and colorimetric method
	Soya based				3.9	< 0.4	
Braun et al. 2010	Bovine milk based	27.0-38.0	35.0		65.0-77.0	32.0-42.0	HPLC-ELSD
Fong et al. 2013	Bovine milk based	61.0-75.0	26.0-46.0	13.0-28.0	63.0-84.0	31.0-82.0	HPLC-MS/MS

Results are presented as interval (minimum and maximum) of all the different infant formulas in the cited references. *Conversion factors of 751 and 770 g mol^{-1} were used for SM and PC, respectively. n.d.: not detected. n.s.: not specified. PE: phosphatidylethanolamine. PI: phosphatidylinositol. PS: phosphatidylserine. PC: phosphatidylcholine. SM: sphingomyelin.

Table 4. Fatty acid composition (%) of individual phospholipids in human milk.

Fatty acid	PE[1]	PI[2]	PS[2]	PC[3]	SM[4]
Capric (C10:0)					0.1
Lauric (C12:0)	0.1-0.6	0.2-1.2	0.1-1.2	0.1-0.4	0.2-0.6
Myristic (C14:0)	0.2-2.4	0.4-3.3	0.1-2.5	0.9-4.5	1.1-2.1
Pentadecylic (C15:0)	0.1-0.2	0.1-0.7	0.1-0.2	0.2-0.4	0.1-0.8
Palmitic (16:0)	**7.2-11.8**	**5.8-17.3**	**7.3-13.4**	**25.1-38.0**	**5.3-21.3**
Palmitoleic (C16:1)	0.5-2.4	0.2-2.1	0.6-2.0	0.4-1.7	0.1-0.7
Margaric (C17:0)	0.2-1.5	0.2-0.7	0.6-1.0	0.3-0.7	0.5-1.4
Heptadecenoic (C17:1)					0.3
Stearic (C18:0)	**23.1-29.1**	**30.6-34.5**	**33.5-42.8**	**16.9-24.7**	**11.8-13.8**
Oleic (C18:1)	**15.8-23.7**	**12.4-20.1**	**15.7-19.4**	**14.0-20.8**	**1.0-4.0**
Linoleic (C18:2)	**13.0-23.8**	**5.3-19.5**	**8.5-23.0**	**13.9-24.1**	**0.3-4.5**
Linolenic (C18:3)	**0.2-4.1**	**0.1-2.5**	**0.1-2.4**	**0.2-1.3**	**0.1-0.7**
Nonadecylic (C19:0)					0.4
Arachidic (C20:0)	0.3-0.4	0.5	0.5	0.25-0.3	**6.4-10.9**
Eicosenoic (C20:1)	1.3-1.4	0.2	0.5	0.4-0.7	0.1-0.5
Eicosadienoic (C20:2)	0.3-1.1	0.2-0.8	0.2-1.4	0.1-0.3	0.6
Eicosatrienoic (C20:3)	1.1-3.5	2.0-5.2	1.3-3.9	0.6-2.4	0.2-03
AA (C20:4)	**4.8-12.7**	**4.5-12.2**	**1.5-4.6**	**1.7-3.3**	**0.3-0.5**
EPA (C20:5)	**0.3-4.2**	**11.7**	**0.5-9.0**	**0.1-2.9**	**0.2-5.3**
Heneicosylic (C21:0)					0.8-2.6
Behenic (C22:0)	0.2			0.2	**12.9-20.7**
Erucic (22:1)	0.1-0.2	0.4	0.5	0.1-0.3	**0.4-11.8**
Docosadienoic (C22:2)	1.5			0.1	4.8
Docosatetraenoic (C22:4)	2.1-3.9	1.4-6.0	1.4-4.2	0.3-0.7	
Docosapentaenoic	0.8-2.4	0.4-2.2	1.6-3.0	0.4-0.9	

(C22:5ω6)					
Docosapentaenoic (C22:5ω3)	0.7-2.3	0.1-0.7	0.5-0.9	0.1-0.2	0.1
DHA (C22:6)	**1.0-5.1**	**0.4-1.7**	**1.5-2.9**	**0.1-0.6**	**0.5-1.1**
Tricosylic (C23:0)					4.0-7.7
Lignoceric (C24:0)	0.3-2.8	0.9	1.2	0.1-0.5	**8.1-19.5**
Nervonic (C24:1)	0.1-0.2	0.5	0.5	0.1-0.7	**9.7-17.7**

Results are presented as interval (minimum and maximum) of all the cited references and including different stages of lactation. Major compounds (~> 10%) or fatty acids of high nutritional relevance appear in boldface. AA: arachidonic acid. EPA: eicosapentaenoic acid. DHA: docosahexaenoic acid. PE: phosphatidylethanolamine. PI: phosphatidylinositol. PS: phosphatidylserine. PC: phosphatidylcholine. SM: sphingomyelin.

[1] Morrison et al. 1967; Bitman et al. 1984; Wang et al. 2000; Benoit et al. 2010

[2]Bitman et al. 1984; Wang et al. 2000

[3] Morrison et al. 1967; Bitman et al. 1984; Wang et al. 2000; Benoit et al. 2010

[4]Morrison et al. 1967; Bitman et al. 1984; Wang et al. 2000; Benoit et al. 2010; Blass et al. 2011

Figure 1. Structure of the main phospholipids found in human milk and infant formulas.

Figure 1. Cilla et al.