Marine oils in infant formulae

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Nutritional requirements for omega-3 fatty acids by infants

The term "infant formulae" refers to foods designed to satisfy the nutritional needs of babies from birth to 4–6 months old who are not being exclusively breastfed. "Follow-on formulae" refer to foods designed for nutrition of babies over 4 months as their main source of liquids and as their diet gradually becomes more versatile. Even though there is presently a consensus in medical circles on the superiority of mother's milk, more and more mothers are using these products for diverse reasons. Therefore, the market for infant formulae is large (over $7 billion per year in the United States). The most rapidly growing segment of this market is formulae supplemented with long-chain poly-unsaturated fatty acids (LC-PUFA).

Human milk is characterized by its unique fatty acid distribution. Even though there are some small variations, all human milks are characterized by the dominance of triacylglycerols (TAG, 98%), glycerophospholipids (1%), and sterols (0.5%), where palmitic acid is predominantly (60–70%) located in the *sn*-2 position of the glycerol backbone. The *sn*-1 and *sn*-3 positions are occupied by unsaturated fatty acids, unlike vegetable oils, cow milk, and infant formulae prepared by blending vegetable oils. During the digestion process, human milk fat (HMF) is hydrolyzed to produce free fatty acids (FFA) and *sn*-2-monoacylglycerols (2-MAG) that are absorbed in the small intestine. Free palmitic acid released from the *sn*-1,3 positions can form insoluble calcium-fatty acid complexes, also referred to as "calcium soaps," which cannot be absorbed in the small intestine and are excreted. This situation causes infants to have lower fatty acid and calcium absorption levels and higher loss of dietary energy. Also, stool hardness, constipation, and even cases of bowel obstruction are reported to occur in infants fed with cow's milk or formulae having TAG where palmitic acid is located mainly at *sn*-1,3 positions. The location of palmitic acid in the TAG of HMF is therefore important with respect to its absorption in infants. Furthermore, HMF provides sufficient quantities of essential linoleic acid

Human milk fat substitutes containing marine oils have the potential to become an important part of infant nutrition.

TABLE 1. Codex Recommendations for LC-PUFA Supplemented Formulae[a]

	Linoleic acid mg/100 kcal	α-Linolenic acid mg/100 kcal	Linoleic/α-linolenic acid ratio	EPA	DHA
IEG of the ESPGHAN and FISPGHAN	Min: 300 Max: 1200 mg	Min: 50 Max: No value	5–15:1	EPA < DHA	<0.5% of total fat

[a] Abbreviations: Codex, Codex Alimentarius Commission; LC-PUFA, long-chain polyunsaturated fatty acids; IED, International Expert Group; ESPGHAN, The European Society for Pediatric Gastroenterology, Hepatology, and Nutrition; FISPGHAN, Federation of International Societies on Pediatric Gastroenterology, Hepatology, and Nutrition.

(LA), α-linoleic acid (ALA), and longer-chain (20- and 22-carbon) PUFA such as arachidonic acid (ARA) and docosahexaenoic acid (DHA). It also contains an adequate amount of omega-3 polyunsaturated fatty acids (n-3 PUFA) such as DHA (0.26%) but very little eicosapentaenoic acid (EPA) (0.07%).

n-3 PUFA are synthesized mainly by uni- and multicellular phytoplankton and algae. Fish fed on algae and marine mammals eating the fish become enriched in n-3 PUFA. The flesh of fatty fish such as herring, mackerel, menhaden, salmon, anchovy, tuna; the liver of lean white fish such as cod and halibut; and the blubber of marine mammals such as seals and whales are the best sources of n-3 PUFA. These acids have been claimed to be very important for the development and growth of infants with respect to brain development and cognitive function (learning ability) as well as in photoreception (vision), since they are found to accumulate in the brain and eye of the fetus, especially during the last trimester of pregnancy.

The supplementation of infant formulae with n-3 LC-PUFA such as EPA, DHA, ALA, and with n-6 LC-PUFA such as ARA has attracted great interest because an adequate supply of LC-PUFA is important for infants during the first year after birth and because the number of breast-feeding women is decreasing. Inadequate supplies of LC-PUFA during nervous system development are of concern because of possible long-term changes in learning ability and reduced visual function. Babies cannot synthesize these fatty acids, but they are found in HMF. Blood levels of these fatty acids are typically higher in breast-fed infants than in infants fed formulae not containing these fatty acids. Infants fed with formulae lacking LC-PUFA have also been reported to have lower DHA levels in the phospholipids of their cerebral cortex than infants fed human milk. Since term infants have a limited capacity to desaturate fatty acids and as a consequence to synthesize LC-PUFA such as ARA, DHA, and EPA, they rely on the dietary supply of these fatty acids during the first months of their lives.

Regulatory recommendations

The supplementation of LC-PUFA into infant formulae is supported by FAO/WHO (Food and Agriculture Organization/World Health Organization) and also allowed by respective EU (European Union) regulations in view of their beneficial effects. The Codex Committee on Nutrition and Foods for Special Dietary Uses requested The Committee on Nutrition of the ESPGHAN (The European Society for Pediatric Gastroenterology, Hepatology and Nutrition) to initiate a consultation process to provide a proposal on nutrient levels in infant formulae. In response to this request, ESPGHAN, together with its sister societies in the global Federation of International Societies on Pediatric Gastroenterology, Hepatology, and Nutrition (FISPGHAN), formed an International Expert Group (IEG) composed of qualified experts in the nutrition area. This group has proposed compositional requirements of infant formula as well as optimal ingredient levels as given in Table 1 (*J. Pediatr. Gastroenterol. Nutr. 41*:584–599, 2005).

As can be seen from Table 1, no upper limit for α-linolenic acid is set since a ratio of linoleic/α-linolenic acid in the range of 5–15:1 is being recommended, which limits the content of ALA. The addition of DHA should not exceed 0.5% of total fat intake, whereas ARA contents should reach at least the same concentrations as DHA, and the content of EPA should not exceed the content of DHA in the infant formulae.

The respective EU legislation, such as the EU Directive 96/4/EEC amending Directive 91/321/EEC, (netlink: www.fsai.ie/legislation/food/legislation_foodnutritional.asp) allows the addition of LC-PUFA at the limits given in Table 2.

The levels of DHA and ARA in commercially available infant formulae, produced by different manufacturers for term and preterm infants, range between 8–19 mg/100 calories and 22–34 mg/100 calories, respectively. The adequate intake of AA, DHA, and EPA for infant formula/diet is 0.50, 0.35, and <0.10%, respectively. It was indicated that higher amounts of EPA in infant formulae may antagonize AA and interfere with infant growth.

Strategies for supplementation with n-3 PUFA

EPA (20:5n-3) and DHA (22:6n-3) are two major omega-3 PUFA that are commercially available from marine oils. Recent developments in food technology such as encapsulation allow fortification

TABLE 2. EU Directives for LC-PUFA-Supplemented Formulae

	n-3 LC-PUFA	n-6 LC-PUFA	EPA
EU Legislation	<1% of total fat	<2% of total fat (1% of the total fat content for AA)	EPA < DHA

of foods with marine n-3 PUFA, without the undesirable fish odor or taste. Two different strategies for supplementation are possible: direct addition of n-3 PUFA concentrates of marine oils to the infant formulae or incorporation of n-3 PUFAs into the HMF substitutes (HMFS), which can be tailor-made by enzymatic interesterification as we reported in the *Journal of Agricultural and Food Chemistry* (*54*:3717–3722, 2006). In Europe, Asia, Canada, and the United States, some commercially available infant formulae for term and preterm infants already contain LC-PUFA such as DHA and ARA. Most of these contain DHA-rich single cell oil (DHASCO; Martek Biosciences Corp., Columbia, Maryland, USA) as the n-3 source, which refers to a mixture of oil extracted from the microalgal species *Crypthecodinium cohnii* and high-oleic sunflower oil. The resulting mixed oil contains 40–45% of product weight as DHA and is marketed as an enrichment ingredient for infant formula (Mead Johnson Nutritionals, Evansville, Indiana, USA), as well as for other functional foods.

HMFS enriched with n-3 fatty acids (both EPA and DHA or DHA alone) may be prepared by enzymatic interesterification or acidolysis reactions using vegetable oils and n-3 fatty acid concentrates. There are numerous approaches to the enzymatic methods available in the literature, although not many commercial scale productions exist. Betapol® produced by Loders Croklaan, Glen Ellyn, Illinois, USA, is an example of commercialized HMFS prepared with lipases through a reaction between tripalmitin and unsaturated fatty acids, but it does not contain n-3 PUFA.

We have investigated the possibilities of producing HMFS that contain n-3 PUFAs as structured lipid (SL) from tripalmitin, hazelnut oil fatty acids, and n-3 PUFA concentrate obtained from menhaden oil by enzymatic interesterification. We succeeded in obtaining a HMFS containing 45.5% palmitic, 37.5% oleic, 4.4% linoleic, and 6.2% EPA plus DHA with 76.6% of palmitic acid at the *sn*-2 position, which is very close to the fatty acid distribution in HMF as reported in our previously cited paper. Based on our research findings, we propose that the SL that contain palmitic acid predominantly at the *sn*-2 position, and that are further enriched with DHA, EPA, and other n-3 PUFA from marine sources will preferably be used in infant formulae to mimic the physical and chemical structure of human milk fat, as well as to provide health benefits associated with these fatty acids. This product has the potential to become an important ingredient for infant nutrition and development if used in commercial infant formulae.

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