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Red blood cell membrane fatty acid composition in infants fed formulas with different lipid profiles

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ABSTRACT

Background: There is growing interest in the fatty acid composition of breast milk and substitute formulas used to replace or complement infant breastfeeding.
Aim: The aims of this study were to assess the impact of two follow-up infant formulas based on cow milk fat, vegetable oils and different docosahexaenoic (DHA) and arachidonic (ARA) acid content on red blood cell membrane fatty acid composition, and determine the percent saturated fatty acid (SFA) incorporation into the membrane.
Study design: This was a double-blind, randomized, controlled, parallel-group clinical trial. Infants received treatment or control product for at least four months before the age of six months. The control group (n = 25) received standard infant formula (FA) and the treatment group (n = 24) received the same formula supplemented with higher DHA and ARA content (FB). The reference group (n = 47) consisted of normal healthy exclusively breastfed infants.
Outcome measure: Red blood cell membrane fatty acid composition was determined by capillary gas chromatography.
Results: Ninety-six infants completed the study (FA, 25; FB, 24; reference, 47). Higher DHA content reflected higher DHA percentage in the red blood cell membrane. Breast milk and FB did not show any significant differences in DHA content. ARA percentage was higher in breastfed infants and palmitic acid percentage was higher in FB- compared with FA-fed infants.
Conclusion: DHA and palmitic acid percent distributions were higher in the red blood cell membrane of infants receiving FB. DHA percent distribution was not significantly different in FB-fed and breastfed infants. SFA percent distribution was not significantly different when comparing both formulas with breast milk.

1. Introduction

In the last decades, there has been growing concern about the fatty acid composition of infant formulas, which are normally used when breastfeeding must be either replaced or complemented following medical advice. Breast milk is the main reference for infant feeding, since it has the best concentration of nutrients and bioactive factors [1].

The fetus, the newborn and breast-fed infants must receive sufficient amounts of long-chain polyunsaturated fatty acids (LCPUFA) to sustain optimal visual and cognitive development. In this sense, breast milk is highly recommended as a source of LCPUFA. When breastfeeding is not possible, the current recommendation is the provision of formulas with adequate docosahexaenoic acid (DHA) and arachidonic acid (ARA) levels [2].

Studies supporting the advantages of breastfeeding have focused on the concentration of LCPUFA in human breast milk, particularly DHA and ARA [3]. These two account for 20% of the brain fatty acid content and are involved in neurodevelopment, promoting healthy neuronal growth, repair and myelination [4]. At the end of pregnancy, ARA is the predominant PUFA in the brain, but DHA accretion after birth makes it the main PUFA in the adult brain, representing approximately 50–60% of the brain's dry weight [5].

Breast milk fat is better absorbed than infant formula fat. Despite similarities in their fatty acid composition, the structure of human milk triacylgycerols is different. Palmitic acid (hexadecanoic acid) is one of the main components of maternal milk, representing around 25% of the lipid profile, from which 60–85% is in sn-2 position of triacylglycerols [6,7]. Addition of palm oil (high palmitic acid content) to

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infant formulas results in a lipid composition closer to that of maternal milk. However, a critical aspect of infant formulas to mimic human milk composition is the bioavailability of nutrients, in this case, fatty acids [8–10].

Triacylglycerols (palmitic acid in sn-2 position) in human milk are digested by pancreatic lipase, which hydrolyzes them on the 1 and 3 carbons and releases saturated fatty acids (SFA), both palmitic and stearic acid, as sn-2-monoacylglycerols. These will later form mixed micelles with bile salts to be absorbed [8]. On the other hand, the greatest part of palmitic acid from palm oil used in infant formulas is present in sn-1 and sn-3 positions of triacylglycerol [11,12], becoming free fatty acid after hydrolysis. Free fatty acids bind to calcium in a process of saponification to form a non-absorbable complex that contributes to the low absorption of fat and calcium, lower weight gain and hardening of stools. The effect of palm oil has been shown in term and pre-term babies and experimental animals [12–14]. In cow milk, the concentration of palmitic acid in sn-2 position is above 45% fat [15].

The role of SFA in milk and breast fat has been recently reviewed, with an emphasis on their relevance and benefits [16]. More recently, focusing in palmitic acid, SFA have been reported as an essential component of membrane, secretory and transport lipids, with crucial roles in protein palmitoylation and signal molecules [17]. Further, the author raises some questions, namely, why the human fetus synthesizes and accumulates 16:0, and the human mammary gland invests in specific pathways to provide the infant with 16:0, proposing that those questions are important for developmental biology, with broad implications for the nutritional care of infants that involve fatty acids that alter sources of metabolic energy, and the growing tissue and plasma lipids [17].

We analyzed the percentage of palmitic acid at sn-2 position of triacylglycerols in infant formulas from the Argentinean market that are used as breast-milk substitutes during the first six months after delivery. We found that formulas with vegetable oils as basic source of lipids had <15% of palmitic acid at sn-2 position, whereas formulas containing a blend with milk fat had 48% of palmitic acid at sn-2 position [18].

The best approach to study fatty acid incorporation into the tissues, particularly the central nervous system, is through the study of red blood cell membrane fatty acids, which are considered representative of the composition of brain cell membranes [19]. Therefore, it would be important to know whether higher DHA and ARA content in infant formulas results in higher red blood cell membrane fatty acid percentage as well as to determine the percent SFA incorporation into the membrane.

The aim of the present study was to assess the impact of two follow-up infant formulas based on cow milk fat, vegetable oils and different DHA and ARA content on red blood cell membrane fatty acid composition, and to determine the percent incorporation of SFA into the membrane.

2. Patients and methods

2.1. Study design and protocol

Healthy term infants being seen for routine follow-up appointments since the first month of life at the Health Observatory of IDIP (Instituto de Desarrollo e Investigaciones Pediátricas, La Plata's Children Hospital, Buenos Aires, Argentina) were recruited for a double-blind, randomized, controlled, parallel-group clinical trial during December 2012–November 2014.

Both gestational age and birth weight of infants were normal (>37–<42 weeks and ≥2500–<4000 g, respectively). Mothers were also healthy and >18 years of age. Written informed parental consent was obtained for each infant before inclusion.

Exclusion criteria included inadequate height-for-age and weight-for-age indices according to the World Health Organization (WHO) charts; infants with acute infections within 15 days before recruitment; chronic diseases; antibiotic or vitamin treatment; a birth weight <2500 g; pathological fetal and neonatal history; anemia not related with nutrition; diagnosis or ongoing studies of genetic alterations; lack of written informed parental consent and mothers with chronic diseases.

The study protocol was approved by IDIP's Institutional Research Review Board and registered in the Ministry of Health of the province of Buenos Aires. It was performed in accordance with the ethical standards laid down in the 1948 Universal Declaration of Human Rights, the Nüremberg Code and the 1964 Declaration of Helsinski and successive revisions and amendments.

Participants were controlled and enrolled since the first month of life at IDIP's Health Observatory. Breastfed infants were followed-up without any intervention. At the time when mothers independently decided to cease breastfeeding and introduce infant formula, infants were randomly assigned to receive either a standard infant formula (control group, FA) or an infant formula supplemented with DHA and ARA (treatment group, FB) to enhance the levels of both fatty acids. A reference group was formed of infants who continued to be exclusively breastfed up to six months (non-randomized). Random allocation of study participants to the intervention was performed with EPIDAT 3.1 (1:1 allocation ratio). None of the two groups received complementary foods other than formula.

Physical examination included infant weight, length and head circumference measurement at baseline (age 2–4 weeks) and at monthly intervals throughout the intervention until the age of 6 months. These measurements were used to calculate height-for-age, weight-for-age and weight-for-height indices, and plotted into reference Z-score tables based on the WHO Child Growth Standard, using WHO Anthro 2007 Version.

Nutritional status was assessed using anthropometric indices of height-for-age, weight-for-age and weight-for-height; Z-scores were calculated with the EPINUT program in Epi Info (version 3.3, 2004). Stunting was defined as a height-for-age (Z-score), underweight as a weight-for-age (Z-score), and wasting as a weight-for-height (Z-score), on the basis of the WHO Child Growth Standard 2007 Version.

Infants were dropped out of the trial if they were subsequently found to have received non-compliant feeding during the intervention, or to have moved to an unidentified address. Infants on any of the two formulas had to fulfill supplementation for a minimum of 4 months before the age of 6 months and breastfeed no more than once a day. Exclusive breastfeeding had to be sustained until the age of 6 months.

Infant formulas were developed and produced by Sancor CUL and had the following composition: Formula A contained (w/w) 66.81% milk fat, 33.19% vegetable oil (32.44% canola and sunflower 50/50% mixture), and 0.75% oil as source of ARA and DHA, obtained from fungi and microalgae. Formula B contained (w/w) 65.41% milk fat, 34.59% vegetable oil (33.05% canola and sunflower 50/50% mixture), 1.24% oil as source of ARA and DHA obtained from fungi and microalgae, and 0.30% oil with high DHA content from microalgae (which contains palmitic acid).

Data for maternal milk composition were taken from 32 mothers of exclusively breastfed infants. In all cases, samples were taken 3 months after delivery. Breast milk samples taken more than a week before or after the third month were discarded. Table 1 shows the fatty acid composition of supplemented formulas and the breast milk composition of 32 exclusively breastfed infants.

2.2. Breast milk sample collection

Before sample collection, mothers washed their hands and breasts with soap. Milk collection was carried out with a sterile automatic breast milk pumper with a vacuum regulator (mini electric breast pump, MEDELA Inc. USA), polystyrene suction funnels and screw-top bottles adapted to suction funnels for direct collection of milk. The bottles and the suction funnels were autoclaved before use. The milk