Prostaglandins, Leukotrienes and Essential Fatty Acids 91 (2014) 97–103

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Liquid human milk fortifier significantly improves docosahexaenoic and arachidonic acid status in preterm infants ☆

C.L. Berseth [a], C.L. Harris [a], J.L. Wampler [a,*], D.R. Hoffman [b], D.A. Diersen-Schade [c]

[a] Department of Medical Affairs, Clinical Research, Mead Johnson Nutrition, 2400 West Lloyd Expy, Evansville, IN 47721, USA
[b] Retina Foundation of the Southwest, 9900 North Central Expressway, Dallas, TX 75231, USA
[c] College of Human Ecology, Division of Nutritional Sciences, Cornell University, Savage Hall, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 19 December 2013
Received in revised form
18 March 2014
Accepted 20 March 2014

Keywords:
Human milk fortifier
Preterm infants
Breast feeding

ABSTRACT

We report the fatty acid composition of mother's own human milk from one of the largest US cohorts of lactating mothers of preterm infants. Milk fatty acid data were used as a proxy for intake at enrollment in infants ($n=150$) who received human milk with a powder human milk fortifier (HMF; Control) or liquid HMF [LHMF; provided additional 12 mg docosahexaenoic acid (DHA), 20 mg arachidonic acid (ARA)/100 mL human milk]. Mothers provided milk samples ($n=129$) and reported maternal DHA consumption ($n=128$). Infant blood samples were drawn at study completion (Study Day 28). Human milk and infant PPL fatty acids were analyzed using capillary column gas chromatography.

DHA and ARA were within ranges previously published for US term and preterm human milk. Compared to Control HMF (providing no DHA or ARA), human milk fortified with LHMF significantly increased infant PPL DHA and ARA and improved preterm infant DHA and ARA status.

1. Introduction

Human milk is considered the preferred source of infant nutrition. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommends the addition of supplemental nutrients to human milk to meet preterm infant requirements [1]. Supplementation of human milk with human milk fortifier (HMF) provides calories as well as macro- and micronutrients at levels approximating estimated preterm infant needs [2]. Infants born prematurely have low total body long chain polyunsaturated fatty acid (LCPUFA) stores [3,4] and the docosahexaenoic acid (DHA) and arachidonic acid (ARA) content of preterm human milk may be insufficient to meet increased needs. Therefore the current ESPGHAN standard for preterm infants also recommends fortification of mother's own human milk as necessary to meet high LCPUFA requirements (11–27 mg DHA/100 kcal and 16–39 mg ARA/100 kcal) or use of infant formulas designed for premature infants [1]. Preterm infant DHA and ARA status rapidly declines [5,6] and low whole blood DHA and/or ARA are associated with poor clinical outcomes, such as increased risk of chronic lung disease and late-onset sepsis [6]. Consequently, postnatal delivery of dietary LCPUFAs in the recommended range represents a challenge for those caring for infants receiving human milk or formula.

Preterm formulas have been developed based on worldwide human milk DHA and ARA data in order to provide standardized fortification [7]. Human milk long chain n-3 and n-6 fatty acids can vary at both population and individual levels and depend particularly on maternal dietary pattern and habits [7–12]. For example, analysis of dietary fatty acid intake and plasma n-3 and n-6 fatty acids from pregnant Canadian women demonstrated that dietary DHA intakes in some women during the third trimester were below that necessary for DHA accretion in both fetal and maternal tissues [13]. Western diets with low intake of n-3-rich fish are also reflected in lower maternal blood DHA [7,9]. Human milk ARA concentrations are typically higher, but less variable, than DHA [7]. Consequently, clinical trials of preterm formulas providing 17 mg DHA and 34 mg ARA per 100 kcal as fed (approximately 0.33% and 0.66% of total fatty acids, respectively, for formulas with 5.1 g total fat/100 kcal formula) have demonstrated improved growth and development compared with similar formulas with no LCPUFAs [14,15].

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; HMF, human milk fortifier; IQR, interquartile range; LA, linoleic acid; LHMF, liquid human milk fortifier; LCPUFA, long-chain polyunsaturated fatty acid; PPL, plasma phospholipid

☆The study was funded by the study sponsor, Mead Johnson Nutrition (MJN; Evansville, IN).

* Corresponding author. Tel.: +1 812 453 7627.
E-mail address: jennifer.wampler@mjn.com (J.L. Wampler).

http://dx.doi.org/10.1016/j.plefa.2014.03.002

Given the increased likelihood for suboptimal DHA and ARA status and risk for the associated negative consequences in premature infants, DHA and ARA in a commercially sterile liquid HMF (LHMF; Enfamil® Mead Johnson Nutrition, Evansville, IN) were targeted, when added to "typical" US human milk, to be similar to the levels studied in preterm formulas. Appropriate absorption of preformed DHA and ARA added in preterm infant formula, as measured by metabolic balance studies [16] and from fortified human milk, as measured by increases in blood levels [17] has been previously demonstrated with no evidence that fortifying human milk with these fatty acids negatively affects availability from human milk. We recently demonstrated that fortification with LHMF, formulated to increase the DHA, ARA, total fat, and protein content of human milk, significantly improved preterm infant growth when compared to milk fortified using a powder HMF without DHA or ARA (Control, Mead Johnson Nutrition) [18]. As part of that study, we collected mother's own preterm milk to provide baseline information on unfortified milk and infant plasma samples to examine how dietary provision of a standardized level of DHA and ARA affects preterm infant status. Here we report on the fatty acid composition of preterm human milk from this large US cohort of lactating mothers, as well as LCPUFAs in plasma phospholipids of their infants.

2. Methods

Participants in this study (150 premature infants and their mothers) were recruited for a double-blind, randomized, controlled, prospective study to evaluate growth, tolerance and safety of a new ultra-concentrated LHMF [18]. Briefly, at enrollment the infants were $\leq$30 3/7 weeks gestational age with birth weights $\leq$1250 g, exclusively fed unfortified human milk (mother's own or donor milk) with enteral intake of $\geq$80 mL/kg per day, and had no congenital malformations or underlying disease likely to interfere with growth or tolerance of fortified milk. For 28 days, or until hospital discharge or discontinuation of breast milk feedings, infants received human milk (mother's own or donor milk) supplemented with either a powder HMF (Control) or the LHMF beginning at half strength on Study Day 1 and full strength thereafter. The study was conducted at 14 sites from 7 states (Florida, Nebraska, North Carolina, Illinois, Arkansas, New York, and Virginia). A parent or guardian of each infant provided written informed consent. Institutional review boards at each study site reviewed and approved the study protocol. Medically-confirmed serious adverse events were monitored and documented throughout the study. At study enrollment, data on infant birth characteristics were collected [18]. In addition, mothers were asked if they regularly ($\geq$1 time per week) consumed any fish and/or DHA supplements during the last 12 weeks of pregnancy. Responses were recorded as "yes" or "no."

2.1. Study fortifiers

Preterm human milk fortified with LHMF provides ~20% more protein and almost double the amount of DHA and ARA when compared with preterm milk fortified with Control HMF. The ranges for DHA and ARA levels naturally observed in human milk support the nutritional acceptability and safety of ranges for the calculated levels of DHA and ARA the LHMF was formulated to provide as well as their estimated ratio in human milk fortified with LHMF. For example, adding LHMF to milk corresponding to the lowest mean US values (0.15% DHA and 0.4% ARA[7]) would increase levels in fortified milk to approximately 0.29% DHA and 0.57% ARA (or 18 mg DHA and 35 mg ARA per 100 kcal; assuming total fat of 3.5 g/100 mL in unfortified preterm human milk and 6.1 g/100 kcal in milk fortified with LHMF). Addition of LHMF to human milk with as much as 1% DHA and/or ARA would lower the level of the LCPUFAs as a percent of total fat (to 0.8% DHA and 0.94% ARA) in fortified human milk. Using the reported values for worldwide means for DHA and ARA, Table 1 shows the calculated macronutrient and polyunsaturated fatty acid (PUFA) estimates for content of preterm milk fortified with study fortifiers at full strength.

Table 1
Estimated macronutrients and PUFA in fortified human milk.

Nutrient	4 Packets powder HMF (Control)+100 mL human milk (per 100 kcal)	4 Vials liquid HMF (LHMF)+100 mL human milk (per 100 kcal)
Protein (g)	3.4	4
Fat (g)	5.6	6.1
Linoleic acid (mg)	730	730
α-Linolenic acid (mg)	54	54
ARA (mg)	20[a]	38
DHA (mg)	13.8[a]	24
Carbohydrate (g)	9.3	7.7

[a] None added; contribution from human milk only. Powder HMF does not include DHA or ARA; Human milk levels calculated using published mean values of 0.32% of total fatty acids for DHA and 0.47% of total fatty acids for ARA [7]. LHMF provides 12 mg DHA and 20 mg ARA when added to 100 ml (66 kcal) human milk.

2.2. Fatty acid analysis

A single milk sample (1–2 ml) was collected from each mother no earlier than postpartum day 6. The samples were collected after the "let down" reflex occurred or following a complete expression of milk to ensure a homogeneous sample. On Study Day 28, blood samples (1 ml) were obtained from the infants and plasma separated from RBCs by centrifugation. Milk and infant plasma samples were frozen at −20 °C and shipped on dry ice to the Retina Foundation of the Southwest (Dallas, TX) for fatty acid analysis. Lipids were extracted, total phospholipids isolated, and fatty acid methyl esters analyzed by capillary column gas chromatography as previously described [19]. Fatty acids in milk and infant plasma phospholipids (PPLs) were expressed as weight percent of total fatty acids. No donor milk was analyzed.

2.3. Sample size determination and statistical methods

A sample size of 50 infants per study group was originally determined adequate to detect group differences in growth [18]. Fisher's exact test was used to analyze maternal consumption of fish and/or use of DHA supplements during the last 12 weeks of pregnancy. The Kruskal–Wallis test was used to analyze the fatty acid content of breast milk and infant plasma phospholipids. Infant plasma fatty acid analyses were conducted for two data sets. A primary analysis included data from all infants who had a blood sample drawn no more than 3 days after the last feeding of human milk fortified with study HMF. A secondary analysis was based on a subset of infants included in the primary analysis who met the following additional criteria (per-protocol efficacy analysis): received $\geq$80% of their energy intake from fortified human milk for at least the first two weeks of the study and were NPO (*nil per os*) for no more than 2 days during this period, consumed study fortifier at full strength on Study Day 3, received no glucocorticoid therapy during the full 28 day study, and had no disease or illness that affected growth.