Docosahexaenoic and Arachidonic Acid Levels in Extremely Low Birth Weight Infants with Prolonged Exposure to Intravenous Lipids

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Objective To report changes in red blood cell long-chain polyunsaturated fatty acids levels in extremely low birth weight (ELBW) infants relative to duration of intravenous lipid emulsion.

Study design Serial blood samples were collected from 26 ELBW infants during the first 2 months of life in the neonatal intensive care unit using a prospective cohort study design. The primary outcome was the change in long-chain polyunsaturated fatty acids levels over the study period relative to a duration of intravenous lipid emulsion of either ≤28 days or >28 days. Secondary outcomes included parenteral and enteral nutritional exposures as well as prematurity-associated morbidities. Longitudinal regression estimated changes in fatty acid levels between the 2 exposure groups.

Results Infants with >28 days intravenous lipid emulsion had 36 more days of intravenous lipid emulsion than did those with ≤28 days ($P < .001$). Docosahexaenoic acid significantly decreased over time in all infants and decreased significantly more in infants exposed to intravenous lipid emulsion for >28 days ($P = .03$). Arachidonic acid significantly decreased over the study period but the decrease was not related to intravenous lipid emulsion duration. Linoleic and α-linolenic acids had significantly larger increases over time in those with longer exposure to intravenous lipid emulsion ($P < .01$).

Conclusion Docosahexaenoic acid status of ELBW infants declined significantly in the first 2 months of life and the decline was significantly greater in those exposed to intravenous lipid emulsion >28 days compared with those exposed ≤28 days. *(J Pediatr 2013;162:56-61)*.

Premature infants in the neonatal period have low red blood cell (RBC) levels of the long-chain polyunsaturated fatty acids (LCPUFA) docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). Low RBC levels, a commonly used indicator of body LCPUFA status, are correlated with an increased risk of developmental, respiratory, and infectious morbidities in premature infants.[1-4] These effects are presumed to reflect the localization of DHA and ARA in the central nervous system and their influence on inflammation, with products of omega-3 (n-3) fatty acid (FA) metabolism considered to be anti-inflammatory and those of n-6 FA to be proinflammatory.[5,6]

In pregnancy, the third trimester is the time of greatest accretion of DHA and ARA by the fetus.[7] Placental transfer of these LCPUFA from mother to fetus is in preference over the essential fatty acids (EFA) linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3).[8] Because preterm delivery ends placental FA transfer, the premature neonate is dependent on parenteral and enteral nutritional provisions of FA. Both means of providing nutrients make available large amounts of EFA and either no or less DHA or ARA than is transferred in utero.[8] LA and ALA are precursors to their respective downstream products, ARA and DHA, but the premature neonate's variable endogenous conversion despite being better than that of term neonates [9-12] (as well as low birth stores, increased utilization, and needs for growth) are thought to contribute to the low LCPUFA levels in these patients and highlight the need for their direct supplementation.

Although breast milk and formulas designed for premature infants provide DHA and ARA in varying amounts, intravenous lipid emulsions available for routine use in the United States are devoid of DHA and ARA. Because extremely premature patients may rely on intravenous lipid emulsion for the first weeks of life,[13] they may be at increased risk of DHA and ARA deficiency and the potential consequences of low levels. In some instances, their inability to tolerate enteral nutrition is prolonged, likely increasing the deficit of DHA and ARA.

ALA	α-Linolenic acid	LA	Linoleic acid
ARA	Arachidonic acid	LCPUFA	Long-chain polyunsaturated fatty acids
BPD	Bronchopulmonary dysplasia		
DHA	Docosahexaenoic acid	n-3	Omega-3
EFA	Essential fatty acids	NDI	Neurodevelopmental impairment
ELBW	Extremely low birth weight	NEC	Necrotizing enterocolitis
FA	Fatty acid	NICU	Neonatal intensive care unit
FEN	Full enteral nutrition	RBC	Red blood cell

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We hypothesize that extremely low birth weight (ELBW) infants are at very high risk of LCPUFA deficiency and that those who receive intravenous lipid emulsion for a prolonged period of time are at greatest risk. Because many ELBW infants with necrotizing enterocolitis (NEC), sepsis, and other morbidities do not receive enteral nutrition for several weeks, we speculate that accrued DHA and ARA deficiencies could contribute to the poor growth and development observed in this unique population. We sought to establish normative data and measure changes in LCPUFA levels in ELBW neonates with a range of illness over the first 2 months of life, stratified by duration of intravenous lipid emulsion. Our hypothesis was that infants with a prolonged exposure to intravenous lipid emulsion would have the greatest decline in levels of DHA and ARA.

Methods

Under a prospective cohort study design, neonates with a birth weight <1000 g admitted to a participating center's neonatal intensive care unit (NICU) within 24 hours of life were screened for entry into the study. Enrolled patients had blood sampling performed at 4 time points for assessment of their RBC DHA and ARA concentrations. Exclusion criteria included exposure to any fat source (parenteral or enteral) prior to the first timed blood sampling, parental refusal of consent, suspected inherited disorder of metabolism, or the presence of a major congenital anomaly. Enrollment occurred over a 24-month period beginning March 2009.

The Children's Hospital of Chicago Research Center (institutional review board study No 2009-13656), Northwestern Memorial Hospital (No. 75007), and NorthShore University Health System (No. EH10-403) institutional review boards approved this study. Written informed consent was obtained from parents prior to participation.

The primary outcome was the temporal change in DHA and ARA levels in RBC phospholipids over the first 8 weeks of life as stratified by duration of exposure to intravenous lipid emulsion. Based on assessment of the enrolled subjects, we determined that exposure to intravenous lipid emulsion beyond 28 days of life constituted a clinical extreme[13] and therefore infants were classified and compared based on a longer exposure to intravenous lipid emulsion, in excess of 28 days (lipid emulsion >28 days), relative to shorter exposures (lipid emulsion ≤28 days).

Demographic, clinical, and nutritional data were collected from the medical record. Demographic data included maternal age, race/ethnicity, mode of delivery, receipt of antenatal steroids prior to delivery, infant sex, gestational age at birth (as defined by best obstetric estimate as documented in the medical record), weight, head circumference and length at birth, and whether small for gestational age (birth weight <10% for gestational age).

Severity of illness was indexed using the Score for Neonatal Acute Physiology–Perinatal Extension II.[14] Mortality and rates of prematurity-associated morbidities were collected including bronchopulmonary dysplasia (BPD, defined as supplemental oxygen or positive pressure use at 36 weeks' postmenstrual age), NEC (Bell stage II or higher), sepsis (positive culture from a sterile site and treatment with at least 7 days of antibiotics for that infection), intraventricular hemorrhage and/or presence of periventricular leukomalacia documented on any head ultrasound, patent ductus arteriosus, retinopathy of prematurity requiring laser surgery or intravitreal bevacizumab, cholestasis (direct bilirubin ≥2 mg/dL), RBC transfusion history, and length of stay.

Nutritional Management

In addition to all medical decision-making, provisions of parenteral and enteral nutrition were left to the discretion of the treating physicians. Participating centers initiated a protein and dextrose–containing solution in the study population just after birth. The intravenous lipid emulsion used at participating centers during the study period was Intralipid (20% solution; Fresenius Kabi, Bad Homburg, Germany), which was initiated at a dosage of 0.5-1 g/kg/d prior to 48 hours of life and advanced to a maximal dose of 3 g/kg/d. Enteral nutrition was advanced by increments of 20 mL/kg/d in most cases. Breast milk was the primary source of enteral nutrition, and, if it was not available, a commercial premature formula was used. Formula used in all cases had a DHA content of either 0.25% or 0.33% and an ARA content of either 0.4% or 0.67%, reflecting the 2 sources of formulas designed for the nutritional needs of premature infants available at the time of the study. Donor breast milk was not used.

Nutritional data collected included both the age and dose at initiation of parenteral nutrition, total duration and maximal doses of intravenous lipid emulsion, age at initiating enteral nutrition, age at reaching full enteral nutrition (FEN, ≥110 kcal/kg/d via the enteral route), days nil per os (no enteral nutrition >12 hours in 1 day) after FEN, additional days of intravenous lipid emulsion infusion after FEN, and form(s) of enteral nutrition used.

FA Analysis

Blood sampling occurred at 4 time points: (1) after birth but before exposure to any nutritional lipid source; (2) at 2 weeks of life; (3) at 4 weeks of life; and (4) at 8 weeks of life or at discharge, whichever occurred earlier.

Blood samples (1 mL) were placed in ethylenediaminetetraacetic acid for anticoagulation. They were immediately centrifuged at 4000*g* at 4°C for 10 minutes, and the RBC portion was separated and stored under nitrogen at −80°C or on dry ice in transfer until analysis. RBC lipids were extracted and subfractionated by chromatography, and the phospholipid FAs were derivatized, separated, and quantified with the use of gas chromatography using previously published methods.[15] FA values are reported as weight percent (wt%) of total FA (g/100 g).

A single sample of milk (5 mL) was collected from women who chose to provide milk for their infants. Milk expressed between 14-16 days postpartum was collected and frozen at −80°C until analysis. Milk samples were analyzed as an aggregate and not stratified by lipid duration.