Randomized Controlled Trial of Early Docosahexaenoic Acid and Arachidonic Acid Enteral Supplementation in Very Low Birth Weight Infants

Brandy L. Frost, MD[1,2], Aloka L. Patel, MD[3], Daniel T. Robinson, MD, MSc[4], Carol Lynn Berseth, MD[5], Timothy Cooper, MD[5], and Michael Caplan, MD[1,2]

Objective To determine feasibility of providing a concentrated emulsified long-chain polyunsaturated fatty acids (LCPUFA) supplement to very low birth weight infants, and to evaluate blood LCPUFA concentrations at 2 and 8 weeks of study supplementation.

Study design This prospective, randomized, double-blind, placebo-controlled trial randomized infants to receive (1) LCPUFA-120 (a supplement of 40 mg/kg/day docosahexaenoic acid [DHA] and 80 mg/kg/day arachidonic acid [ARA]; DHA:ARA at 1:2 ratio), (2) LCPUFA-360 (a supplement of 120 mg/kg/day DHA and 240 mg/kg/day ARA), or (3) sunflower oil (placebo control). Infants received supplement daily for 8 weeks or until discharge, whichever came first. Whole blood LCPUFA levels (wt%; g/100 g) were measured at baseline, 2 weeks, and 8 weeks.

Results Infants were 28 weeks of gestation (IQR, 27-30 weeks of gestation) and weighed 1040 g (IQR, 910-1245 g). At 2 weeks, the change in blood DHA (wt%) from baseline differed significantly among groups (sunflower oil, n = 6; −0.63 [IQR, −0.96 to −0.55]; LCPUFA-120: n = 12; −0.14 [IQR, −0.72 to −0.26]; LCPUFA-360, n = 12; 0.46 [IQR, 0.17-0.81]; P = .002 across groups). Change in blood ARA (wt%) also differed by group (sunflower oil: −2.2 [IQR, −3.9 to −1.7]; LCPUFA-120: 0.1 [IQR, −2.1 to 1.1] vs LCPUFA-360: 2.9 IQR, 1.5 to 4.5]; P = .0002). Change from baseline to 8 weeks significantly differed between groups for DHA (P = .02) and ARA (P = .003).

Conclusions Enteral LCPUFA supplementation supported higher blood DHA by 2 weeks. LCPUFA supplementation at 360 mg of combined DHA and ARA is likely necessary to reduce declines as well as allow increases in whole blood concentrations in the first 8 weeks of life. *(J Pediatr 2021;232:23-30)*.

Trial registration Clinicaltrials.gov: NCT03192839

Providing sufficient macronutrients and micronutrients to preterm infants has the potential to improve neurodevelopment.[1-3] Long-chain polyunsaturated fatty acids (LCPUFA) are important to infant eye and brain development.[4-8] Data suggest supplementation of docosahexaenoic acid (DHA, 22:6n-3) may improve indicators of visual function in preterm infants.[9] Low blood LCPUFA levels have been associated with risk of neonatal morbidities, such as bronchopulmonary dysplasia (BPD), yet supplementation of DHA alone (no added arachidonic acid [ARA]) in preterm infants increased the risk of BPD.[10,11] A recent meta-analysis suggested that LCPUFA supplementation may contribute to a reduction in BPD as well as necrotizing enterocolitis (NEC).[12]

Several factors contribute to the deficiencies in LCPUFA that develop in preterm infants. The third trimester is the time of maximal transplacental transfer of LCPUFA, including DHA and ARA (20:4n-6), and accretion by the fetus. Preterm infants do not benefit from this time of maximal transfer. Blood LCPUFA levels at 1 week of age correlate with gestational age, meaning that most premature infants have significantly lower LCPUFA levels compared with those born full term.[13] Furthermore, the most premature infants may take weeks to attain full enteral feeds, and at present, most infants receive LCPUFA only via enteral feeds. Importantly, DHA concentrations in North American mother's own milk are variable and DHA in donor human milk has been demonstrated as lower compared with mother's own milk.[14,15] Although intravenous lipid formulations

ARA	Arachidonic acid
BPD	Bronchopulmonary dysplasia
DHA	Docosahexaenoic acid
LCPUFA	Long-chain polyunsaturated fatty acids
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PUFA	Polyunsaturated fatty acids

From the [1]Department of Pediatrics, NorthShore University HealthSystem, Evanston; [2]Department of Pediatrics, University of Chicago Pritzker School of Medicine; [3]Department of Pediatrics, Rush University Medical Center; [4]Department of Pediatrics, Northwestern University Feinberg School of Medicine, Chicago, IL; and the [5]Clinical Research, Department of Medical Affairs, Mead Johnson Nutrition, Evansville, IN

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that have LCPUFAs are increasingly available, ideally the preterm infant's primary source of nutrition should be provided via the enteral route.[16]

Extremely preterm infants experience declines in LCPUFA over the first 2 weeks of life while being nourished largely via parenteral nutrition.[17] Maternal and preterm infant LCPUFA supplementation has been investigated using different methods.[11,18-23] Previously, we enrolled extremely low birth weight infants in a placebo-controlled, double-blind, randomized trial using a concentrated LCPUFA supplement applied to the buccal mucosa.[24] This method of administration did not alter blood LCPUFA. Therefore, our objective in the current trial was to determine the feasibility of using a concentrated emulsified LCPUFA supplement via the enteral route to very low birth weight infants (at 2 concentrations of DHA and ARA). Blood LCPUFA were evaluated at 2 weeks and 8 weeks of supplementation.

Methods

This study was conducted in 2 Chicago metropolitan area level III neonatal intensive care units (NICUs) (NorthShore University Health System and Rush University Medical Center). After obtaining informed consent from the parent or guardian, very low birth weight infants were enrolled into this prospective, randomized, double-blind, placebo-controlled trial within the first 72 hours of life. Exclusion criteria included having a known metabolic disorder, having a congenital gastrointestinal anomaly, being deemed inappropriate for the study by the treating physician, or being in another investigational therapy. The first patient was enrolled in August 2017. Study supplement was completed for the final infant in August 2018.

Enrolled infants were assigned to one of 3 study groups via computer generated randomization: (1) LCPUFA-120 (40 mg/kg/day DHA and 80 mg/kg/day ARA), (2) LCPUFA-360 (120 mg/kg/day DHA and 240 mg/kg/day ARA), or (3) sunflower oil (placebo control). The DHA:ARA ratio was 1:2 for the LCPUFA-120 and LCPUFA-360 study supplements. The LCPUFA-120 supplement was selected because it approximates both the in utero LCPUFA accretion rates, as well as current supplementation strategies in preterm formula. Furthermore, LCPUFA supplementation in preterm formula targets average human milk LCPUFA content, recognizing that maternal milk levels range widely. In addition, provision of ARA in addition to DHA has been recommended by consensus reports.[25] To provide a robust contrast with the LCPUFA-120 supplement, and to overcome any concerns regarding absorption or difficulties with the substance adhering to tubing, we selected the LCPUFA-360 supplement. Randomization ratio was 2:2:1 for LCPUFA-120:LCPUFA-360:control in blocks of 5. Study supplements were barcoded to manage and verify administration. A hospital pharmacist randomized patients and prepared the study supplement or sunflower oil control using amber syringes to maintain blinding of the study team, clinical team, and family of the infant. The supplement was given via nasogastric tube or orally with a feed when infants were taking everything orally. Specifically, the supplement would be administered via the nasogastric tube just before a feed; thus, the feeding was used as a flush to follow the supplement. If the infant was not being fed enterally, the supplement could be flushed with sterile water via the nasogastric tube. The nursing staff administered the supplement at regular intervals throughout each study day, provided as 3-4 daily doses. Supplementation continued for up to 8 weeks or discharge, whichever occurred first.

The medical charts of both infants and mothers were reviewed for baseline maternal and infant demographics, maternal medical and pregnancy history, maternal polyunsaturated fatty acids (PUFA) supplementation in both pregnancy and lactation, and measures of severity of illness for neonates (Score for Neonatal Acute Physiology-Perinatal Extension II).[26] The clinical outcomes recorded included respiratory distress syndrome, intraventricular hemorrhage (any grade), periventricular leukomalacia, patent ductus arteriosus, spontaneous intestinal perforation, NEC, BPD defined as requiring supplemental oxygen at 36 weeks postmenstrual age, retinopathy of prematurity requiring treatment, late-onset sepsis (positive and negative culture), cholestasis, and mortality.

Measures of nutritional intake included parenteral nutrition prescriptions, timing of initiation of feedings, and time to full enteral nutrition (defined as 120 kcal/kg/day). Prescribing of nutritional provisions was at the discretion of the treating neonatologist. In general, parenteral nutrition was started in both NICUs within the first day of life. Infants received intravenous lipid emulsion with Intralipid 20% solution (Fresenius Kabi) within 48 hours of age. Enteral feedings were initiated when deemed appropriate at 20 mL/kg/day or less and advanced as tolerated. Human milk was provided using either mother's own milk or donor human milk if mother's own milk was unavailable. The donor human milk was obtained from the same milk bank for both NICUs. Donor human milk was transitioned to preterm formula at 33 weeks postmenstrual age at Rush University Medical Center, and after 4 weeks of age at NorthShore University HealthSystem.

Serial milk samples, including mother's own milk and/or donor human milk, were obtained to measure the LCPUFA content of enteral nutrition. Samples were collected on dried filter paper at 1, 2, 4, and 8 weeks of age or at discharge if the infant was receiving human milk feedings at that time. Whole blood samples were obtained as capillary blood spots to measure the blood LCPUFA levels at baseline (before starting supplements), 2 weeks, and 8 weeks of supplementation. For LCPUFA analysis, whole blood and human milk samples were collected on Perkin Elmer 226 filter paper (pretreated with a 3-component antioxidant cocktail) and allowed to dry at room temperature for 15 minutes. Filter papers were mailed to OmegaQuant for analysis. One punch of the dried spot was transferred to a screw-cap glass vial followed by addition of boron trifluoride in methanol, toluene, and

methanol. It was shaken and then heated at 100°C for 45 minutes. Hexane and distilled water were added after the mixture cooled. After vortexing briefly, the samples were spun to separate layers and an aliquot of the hexane layer that contained the fatty acid methyl esters was transferred to a vial for gas chromatography. Gas chromatography flame ionization detection was carried out using a GC2010 Gas Chromatograph (Shimadzu Corporation) equipped with a SP2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 μm film thickness; Supelco) using hydrogen as a carrier gas. Fatty acids were identified by comparison with a standard mixture of fatty acids characteristic of red blood cells (GLC OQ-A, NuCheck Prep; this mixture was also used to construct individual fatty acid calibration curves). Whole blood fatty acids are reported as weight% (wt%) of total fatty acids (g/100 g). This method of measuring fatty acid levels has been previously described for both blood spots and milk spots.[27-29]

Statistical Analyses

The primary outcome for this study was blood LCPUFA levels at 2 weeks. Secondary outcomes were blood LCPUFA levels at 8 weeks, days to reach full enteral feeds (defined as 120 kcal/kg/day), and the incidence of NEC and BPD. Data are presented as median (IQR) or number (%). Nonparametric approaches including the Kruskal-Wallis and Fisher exact tests were used to compare clinical characteristics and blood fatty acid concentrations in cross-sectional comparisons. The Wilcoxon rank-sum test was used to compare fatty acid concentrations in milk by participating center. Linear regression accounted for repeated fatty acid concentrations in each infant and identified whether fatty acid changes differed by group longitudinally. Reported beta coefficients reflected change in fatty acid concentrations for the LCPUFA-120 and LCPUFA-360 groups when compared with the sunflower oil group as reference, adjusted for relevant covariates, throughout the 8 weeks of supplementation. Given the biological relevance of sex and gestational age as a reflection of duration of fetal accretion, these variables were included in the multivariable model as well as study center.[17,30] Analyses were performed using Stata v12.1 (StataCorp). We aimed to enroll 30 infants into the study, with 12 per each of the LCPUFA groups, and 6 in the placebo; a power analysis was not performed.

Results

Between July 2017 and August 2018, 30 infants were enrolled (**Figure 1**). No significant group differences were detected in baseline demographic and nutritional outcomes (**Table I**). The age at which intravenous lipids were discontinued and age at full enteral nutrition were similar among groups (**Table I**). All infants survived to discharge and morbidities of prematurity were similar across the groups (**Table I**). Of the 30 infants, 16 (53%) received a blood transfusion during the study, with no difference among groups (P = .06). One infant in the LCPUFA-120 group developed NEC and did not receive the study supplement during the period of bowel rest. All other infants received the assigned study supplement daily and displayed no indicators of intolerance to the supplement itself or administration with enteral feeds. Human milk LCPUFA concentrations were similar when comparing centers, aside from DHA in the first milk samples and eicosapentaenoic acid in the week 8/discharge samples (**Table II**; available at www.jpeds.com).

Infants received study supplementation from 2 days of age (IQR, 2-3 days of age) through 55 days of age (IQR, 45-56 days of age). Blood samples for LCPUFA analysis were drawn on days of age 2 (IQR, 2, 3 of age), 15 days of age (IQR, 14, 17 days of age), and 53 days of age (IQR, 46, 55 days of age). The baseline blood LCPUFA levels (DHA, ARA, linoleic acid, alpha-linolenic acid, and eicosapentaenoic acid) were similar among groups (**Table III**). At 2 weeks, the blood DHA levels were significantly different between groups. ARA and eicosapentaenoic acid were also significantly different between groups with higher levels in the LCPUFA-360 group. At 8 weeks, these differences in ARA and DHA persisted.

The absolute changes in blood DHA levels included a change from baseline by −0.63 wt% (IQR, −0.96 to −0.55 wt%) during the first 2 weeks in the sunflower oil group, and from baseline over the 8 weeks by −0.82 wt% (IQR, −1.14 to −0.6 wt%) in that group (**Figure 2**, A). In the LCPUFA-120 group, the absolute changes in DHA were–0.14 wt% (IQR, −0.72 to 0.26 wt%) over the first 2 weeks and an increase of 0.34 wt% (IQR, 0.1 to1.0 wt%) over the 8 weeks. In contrast, the LCPUFA-360 group demonstrated a 0.46 wt% (IQR, 0.17 to 0.81 wt%) increase in DHA at 2 weeks and an increase of 0.6 wt% (IQR, 0.29 to 1.32 wt %) at 8 weeks. These absolute changes were significantly different between groups during the first 2 weeks (P = .002) and during the 8 weeks (P = .021). Similarly, blood ARA levels decreased and showed an absolute change in concentration of −2.2 wt% (IQR, −3.9 to −1.7 wt%) in the sunflower oil group, a change of 0.099 wt% (IQR, −2.1 to 1.1 wt%) in the LCPUFA-120 group, and an increase of 2.9 wt% (IQR, 1.5 to 4.5 wt%) in the LCPUFA-360 group (P = .0002 between groups) by 2 weeks (**Figure 2**, B). ARA demonstrated a change of −4.2 wt% (IQR, −5.5 to – 2.4 wt%) in the sunflower oil group, a change of −0.16 wt% (IQR, −0.12 to 1.1 wt%) in the LCPUFA-120 group, and increased by 2.02 wt% (IQR, 0.78 to 3.17 wt%) in the LCPUFA-360 group over 8 weeks (P = .003 between groups).

The baseline infant blood DHA levels were notably higher at one center compared with the other: 3.8 wt% (IQR, 3.5-4.2 wt%) vs 2.9 wt% (IQR, 2.4-3.5 wt%) (P = .002). There was no significant difference in maternal use of dietary PUFA supplements during pregnancy between centers.

Multivariable models identified group assignment and study center as significant predictors of change in blood DHA and ARA levels in the LCPUFA groups vs the sunflower oil group over the 8 weeks of study supplementation (**Table IV**).

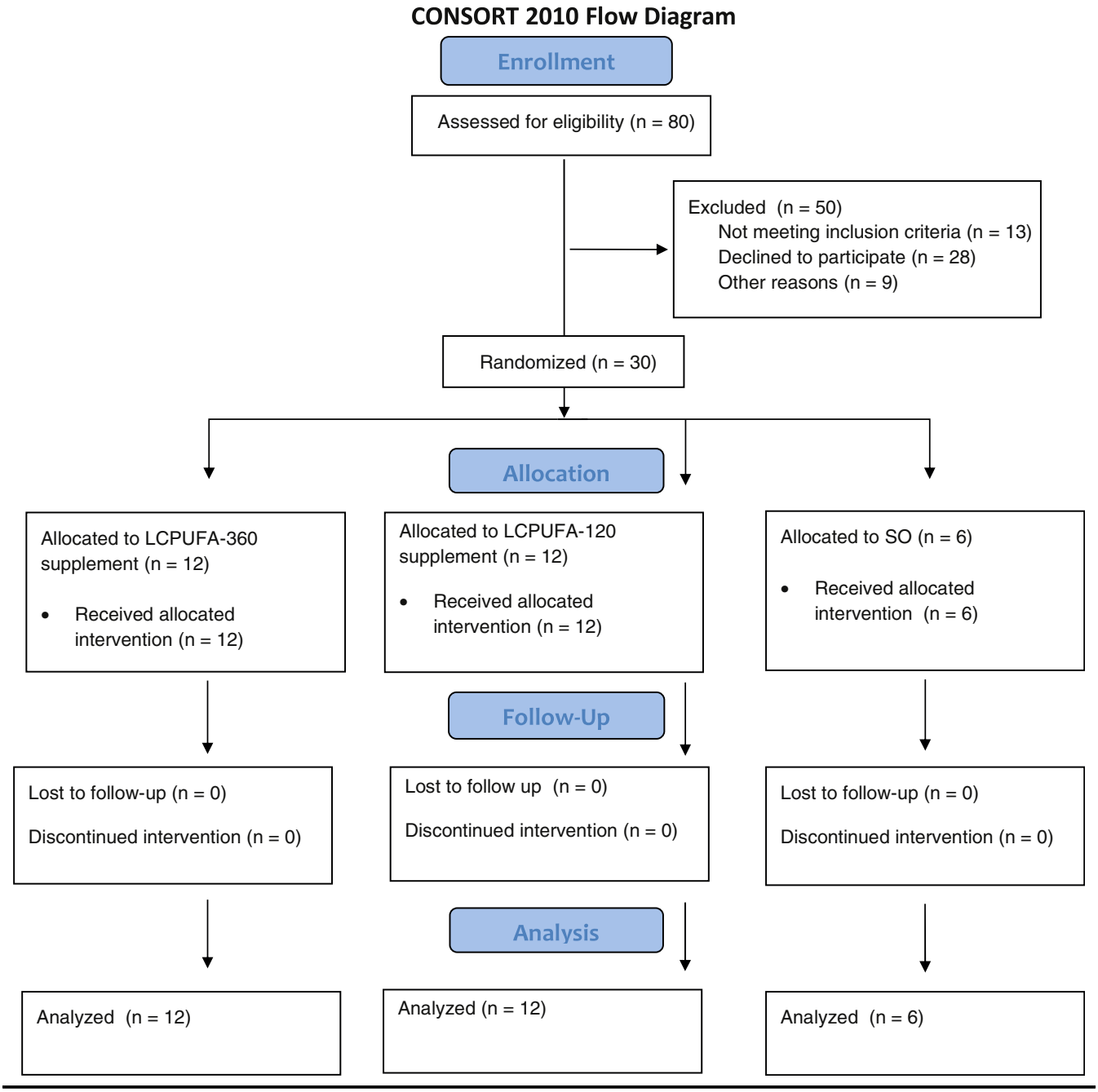

Figure 1. CONSORT diagram depicting enrollment into study, allocation to study group, follow up, and analysis of results.

Discussion

We established the feasibility of providing a concentrated emulsified PUFA supplement as daily nutritional support in 30 very low birth weight neonates. Previously, we demonstrated that blood LCPUFA levels decrease by 2 weeks of age in preterm infants.[17] In the current study, blood DHA levels decreased in infants receiving no supplemental DHA. LCPUFA supplementation at 120 mg of DHA and ARA seemed to maintain a steady DHA status, and blood DHA concentrations increased in infants receiving LCPUFA supplementation at 360 mg of DHA and ARA. Similarly, blood ARA levels decreased in the infants receiving no supplemental LCPUFA, remained similar to baseline levels in infants receiving LCPUFA-120 supplementation, and increased for infants receiving LCPUFA-360 supplementation. Group differences persisted at 8 weeks, with higher blood DHA and ARA levels in infants receiving LCPUFA-360 supplementation. In addition, the supplement was generally well-tolerated by all infants.

Table I. Maternal and infant demographics and outcomes

Characteristics	All (n = 30)	Sunflower oil (control) (n = 6)	LCPUFA-120 (n = 12)	LCPUFA-360 (n = 12)	*P* value*
Gestational age, weeks	28 [27-30]	30 [28-31]	28 [26.5-28.5]	29 [27-30.5]	.12
Birthweight, g	1040 [910-1245]	1135 [910-1350]	1070 [945-1282.5]	985 [902.5-1237.5]	.81
Maternal age, years	29.5 [22-33]	33.5 [28-34]	30 [21-35]	29 [22-31]	.22
Maternal supplementation†	8 (27)	4 (67)	3 (25)	2 (17)	.11
Multiple gestation	8 (27)	2 (33)	2 (17)	4 (33)	.65
Completed antenatal steroids	25 (83)	5 (83)	8 (67)	12 (100)	.19
Cesarean delivery	22 (73)	5 (83)	7 (58)	10 (83)	.49
Small for gestational age	10 (33)	4 (67)	2 (17)	4 (33)	.15
Female	18 (60)	6 (100)	6 (50)	6 (50)	.09
SNAPPE-II	18.5 [9-23]	18 [14-22]	14 [9-23.5]	20 [7.5-24.5]	.98
Length of stay, days	61 [49-84]	47.5 [34-62]	58.5 [49-82.5]	71 [52-106.5]	.2
Intravenous lipid infusion started, age, hours	16 [2.5-28]	14.75 [3-31]	2.5 [2-10.5]	26.5 [18-29]	.02
Intravenous lipid infusion stopped, age, days	9.5 [7-9]	11 [9-11]	8.5 [7-14.5]	10.5 [7.5-12]	.77
Parenteral protein infusion started, age, hours	3 [2-4]	3 [3-3]	2 [2-2.75]	4 [3.5-5]	.004
Enteral nutrition initiation, days	1 [0-2]	1 [0-2]	1 [0.5-2]	0 [0-1]	.35
Full enteral nutrition, days	12 [10-14]	13 [12-14]	10 [9-17]	12 [11-13.5]	.34
Respiratory distress syndrome + surfactant	13 (43)	2 (33)	4 (33)	7 (58)	.56
Intraventricular hemorrhage	5 (17)	2 (33)	2 (17)	1 (8)	.39
NEC or spontaneous intestinal perforation	1 (3)	0	1 (8)	0	1
Therapy for retinopathy of prematurity	2 (7)	1 (17)	1 (8)	0	.67
Sepsis	8 (27)	3 (50)	3 (25)	2 (17)	.38
BPD	3 (10)	0	1 (8)	2 (17)	.83
Cholestasis	1 (3)	0	0	1 (8)	1

Values are number (%) or median [IQR].
SNAPPE-II, Score for Neonatal Acute Physiology-Perinatal. Extension II.
**P* values are for comparisons of all groups, evaluated using the Kruskal-Wallis test for continuous variables and Fisher exact for dichotomous variables.
†Unknown (n = 5).

Previous studies have demonstrated the ability to maintain LCPUFA levels using enteral supplementation in preterm infants. In formula that had DHA and ARA added at different concentrations, Clandinin et al described a dose-response relationship using increasing dosage when supplementing formula with DHA and ARA, demonstrating red blood cell membrane LCPUFA levels approximating those of breast fed infants at 6 weeks of age.[31] The manufacturers of formula for preterm infants now routinely add LCPUFA. Furthermore, researchers have also sought to increase breast milk concentration using maternal supplementation strategies, including high-dose LCPUFA supplementation.[21] As compared with published values of LCPUFA concentrations in preterm human milk, we detected higher linoleic acid and ARA along with comparable alpha-linolenic acid and DHA concentrations.[32]

Extremely preterm infants have a decreasing DHA status owing to inadequate enteral feeding volumes for up to several weeks after birth and because LCPUFA-containing intravenous emulsions are not yet in routine use. Several studies have evaluated the use of a concentrated supplement. Infants previously randomized (n = 31) to 1 of 3 different DHA

Table III. Whole blood LCPUFAs measured from baseline through hospital discharge

Fatty acid	All (n = 30)	Sunflower oil (n = 12)	LCPUFA-120 (n = 12)	LCPUFA-360 (n = 12)	*P* value
Baseline					
Linoleic acid, 18:2n-6	10.5 [8.7-12.3]	10.5 [10.2-10.5]	10.2 [7.9- 15.4]	10.3 [8.5-11.7]	.98
Alpha-linolenic acid, 18:3n-3	0.27 [0.2-0.32]	0.26 [0.24-0.29]	0.26 [0.18-0.52]	0.28 [0.23-0.34]	.89
ARA, 20:4n-6	15.4 [13.6-16.2]	15 [13.4-17.5]	15 [13.2-16.4]	15.5 [14-16.2]	.92
Eicosapentaenoic acid, 20:5n-3	0.16 [0.12-0.22]	0.19 [0.13-0.22]	0.14 [0.1-0.16]	0.18 [0.15-22]	.27
DHA, 22:6n-3	3.5 [2.8-4.2]	3.7 [3.1-4]	3 [2.6-3.5]	3.6 [3.1-4.2]	.15
2 Weeks					
Linoleic acid, 18:2n-6	12.3 [10.8-13.8]	14.2 [12.6-15.2]	12.4 [10.9-14.9]	11.3 [9.9-12.6]	.03
Alpha-linolenic acid, 18:3n-3	0.24 [0.22-0.3]	0.24 [0.23-0.24]	0.25 [0.2-0.3]	0.23 [0.22-0.3]	.99
ARA, 20:4n-6	15.1 [14.1-18.4]	12 [11.3-12.7]	14.8 [14.1-15.2]	18.9 [16.8- 19.8]	.0001
Eicosapentaenoic acid, 20:5n-3	0.3 [0.22-0.37]	0.23 [0.19-0.28]	0.28 [0.22-0.36]	0.36 [0.33-0.38]	.04
DHA, 22:6n-3	3.3 [2.8-4]	2.8 [2.3-3.1]	3.1 [2.4-3.4]	4.1 [3.9-4.3]	.0001
8 Weeks					
Linoleic acid, 18:2n-6	18.6 [15.8-21]	21.9 [20.3-22.4]	15.8 [14.1-20.4]	18.1 [16.5-20]	.03
Alpha-linolenic acid, 18:3n-3	0.65 [0.34-0.75]	0.72 [0.59-0.74]	0.42 [0.26-0.67]	0.75 [0.38-0.99]	.06
ARA, 20:4n-6	14.9 [12.8-17]	10 [9.7-11.9]	14.3 [13.9-15.4]	17.1 [15.8-17.9]	.005
Eicosapentaenoic acid, 20:5n-3	0.35 [0.27-0.47]	0.24 [0.18-0.32]	0.34 [0.28-0.41]	0.38 [0.34-0.47]	.05
DHA, 22:6n-3	3.7 [3.1-4.1]	2.6 [2.1-3]	3.5 [3.2-4]	4.1 [3.9-4.9]	.001

Values are reported in units of wt % (g/100 g of fatty acids) and as median [IQR].
P values are for comparisons of all groups, evaluated using the Kruskal-Wallis test.

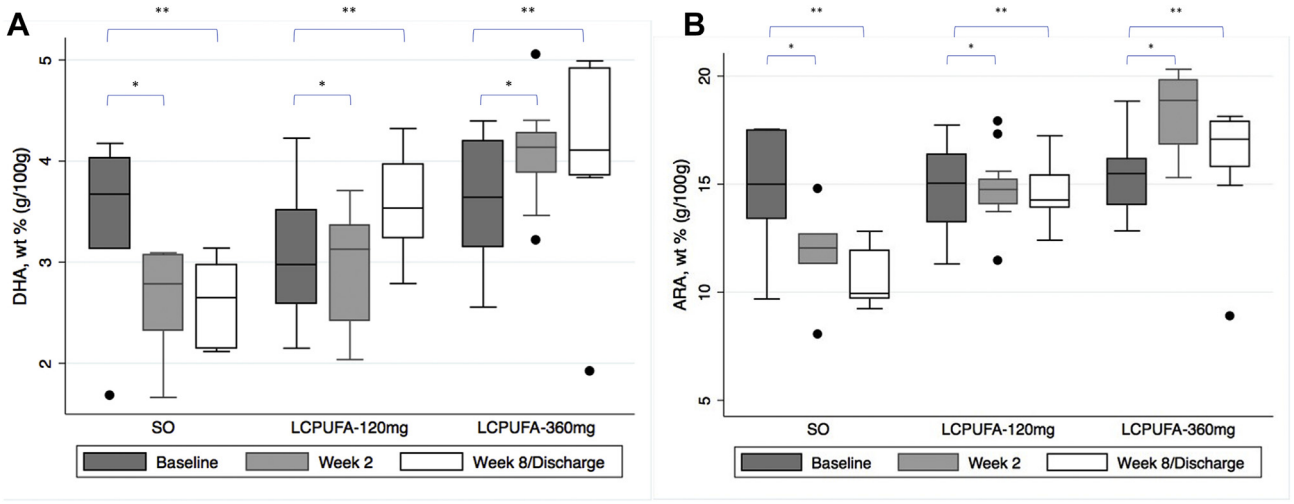

Figure 2. Change in **A,** DHA and **B,** ARA levels during the course of supplementation. The time course of supplementation is shown for all 3 groups, for DHA and ARA levels (levels shown as wt%). For both figures, bars with * indicate differences between groups when comparing absolute changes in levels from baseline to 2 weeks based on Kruskal-Wallis (P = .002 for DHA and P = .0002 for ARA). For both figures, bars with ** indicate differences between groups when comparing absolute changes in levels from baseline to 8 weeks/discharge based on Kruskal-Wallis (P = .021 for DHA and P = .003 for ARA).

concentrations (via emulsion from tuna oil) maintained blood DHA concentrations as well as infants whose mothers took a DHA supplement (but whose infant did not directly receive supplemental DHA).[33] Blood DHA levels decreased only in the group receiving no DHA supplementation. In a separate study, preterm infants of 24-34 weeks gestation (n = 60) randomized to receive 50 mg/day of algal DHA liquid or placebo until discharge or $37^{6/7}$ weeks of gestation were compared with term infants (n = 30) who did not receive supplementation.[34] Blood DHA levels significantly increased over the course of supplementation compared with preterm infants receiving no supplementation; however, blood DHA remained lower in infants receiving supplementation compared with term neonates. In the context of this evidence, along with our results presented here, very low birth weight infants likely require LCPUFA supplementation above that found in maternal milk and preterm formulas to maintain LCPUFA levels that approximate in utero accretion rates.

Table IV. Multivariable regression analyses showing associations between exposures and changes in whole blood DHA and ARA during intervention

Exposure variables	β [95% CI] (wt%)	*P* value
DHA		
LCPUFA-120*	0.54 [0.02 to 1.06]	.04
LCPUFA-360*	0.86 [0.34 to 1.38]	.001
Gestational age	0.06 [−0.03 to 0.15]	.21
Female sex	−0.32 [−0.70 to 0.06]	.097
Study center	−0.58 [−0.98 to −0.18]	.004
ARA		
LCPUFA-120*	2.60 [0.94 to 4.30]	.002
LCPUFA-360*	3.6 [1.9 to 5.3]	<.001
Gestational age	0.02 [−0.28 to 0.33]	.87
Female sex	−0.7 [−1.9 to 0.52]	.26
Study center	−1.5 [−2.8 to −0.16]	.028

*Reference group is placebo.

Although we supplemented the infants in the LCPUFA-360 group with significantly more DHA and ARA than is currently in routine use in formulas, and their blood LCPUFA levels increased compared with the other 2 groups, they remained below levels reported for term infants. Thus, even at the higher level of supplementation, these preterm infants did not seem to be absorbing LCPUFA in excess of normal in utero accretion. Similar findings have been reported previously in preterm infants supplemented with higher doses of DHA in human milk and formula.[35] Although our focus was mainly on feasibility, larger studies in the future may be able to provide more insight into the effect of these higher doses of LCPUFA on important neonatal morbidities and long-term outcomes, given the potential for LCPUFA to modulate inflammation and potential metabolic effects, including growth and adiposity.[36-38]

Our study was not powered to evaluate differences in clinical outcomes. It is plausible that achieving LCPUFA levels approaching term reference would translate into effects on clinical outcomes, but we do not yet have enough data to correlate specific levels with clinical outcomes. Although a recent study found DHA supplementation alone led to increased risk of BPD, no added ARA supplementation was used in that particular study.[11] Furthermore, a maternal supplementation study using DHA alone similarly found an increased risk of BPD in the infants receiving milk from mothers in the supplementation group, compared with those receiving milk from the mothers in the placebo group.[39] The impact of a combined DHA/ARA

enteral supplement merits further study with regard to clinical outcomes in preterm infants.

LCPUFA-containing intravenous lipid emulsions are now being used in NICUs; however it is unclear if these products provide appropriate quantities and balance of LCPUFA.[40,41] Thus, further research is needed on providing very low birth weight infants with adequate LCPUFA supplementation, incorporating both enteral and parenteral strategies.

The limitations of our study were its small size, which precluded assessing the clinical effects of supplementation. The study setting of a Midwestern city may also impact generalizability of our results, because maternal dietary intake of fish is known to impact LCPUFA levels in mother's own milk.[42,43] In fact, although only 20 miles separate the participating centers, infant blood levels at birth were considerably different, as were milk DHA levels in early samples in a consistent manner (ie, lower in blood and milk at the same center). However, consistent concentration-dependent patterns were detected for both DHA and ARA at both study time points, suggesting that the effects were not likely due to potential differences in maternal intake.

In conclusion, it is plausible that maintaining blood LCPUFA levels near in utero accretion rates may be of benefit in this population. Nonetheless, recent trials have demonstrated conflicting results, including risk from DHA supplementation alone with no added ARA.[11] Although clinical practice trends demonstrate earlier feeding initiation in the most premature infants, alternate approaches to providing LCPUFA to preterm infants are needed. In this small feasibility trial, we demonstrated that a concentrated and emulsified LCPUFA supplement can maintain and support higher blood LCPUFA levels in supplemented infants. Further study is needed to identify clinical implications of this combined supplement. ■

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Reprint requests: Brandy L. Frost, MD, NorthShore University HealthSystem, 2650 Ridge Avenue, Walgreen Building Suite 1505, Evanston, IL 60201.
E-mail: bfrost@northshore.org

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Table II. Human milk LCPUFAs from study week 1 through hospital discharge at each center

Fatty acids	Milk source	Week 1*	Week 2†	Week 4‡	Week 8/discharge§
n-6					
Linoleic acid, 18:2n-6	Center 1	18.0 [15.7-18.4]	17.8 [16.5-18.7]	18.1 [17.3-18.4]	21.2 [16.7-21.5]
	Center 2	18.8 [15.3-19.0]	17.2 [16.2-18.2]	18.0 [16.3-20.2]	17.8 [15.3-18.6]
ARA, 20:4n-6	Center 1	0.9 [0.8-1.2]	0.8 [0.8-1.0]	0.7 [0.5-0.8]	0.5 [0.4-0.5]
	Center 2	0.9 [0.7-1.0]	0.8 [0.5-1.1]	0.7 [0.5-0.8]	0.6 [0.5-0.8]
n-3					
Alpha-linolenic acid, 18:3n-3	Center 1	1.3 [0.9-1.5]	1.3 [1.1-1.6]	1.5 [1.5-1.5]	1.7 [1.0-1.9]
	Center2	1.0 [0.9-1.5]	1.3 [1.1-1.6]	1.4 [1.2-1.5]	1.4 [0.9-1.4]
Eicosapentaenoic acid, 20:5n-3	Center 1	0.04 [0.03-0.05]	0.04 [0.03-0.05]	0.03 [0.02-0.05]	0.02 [0.02-0.03]**
	Center 2	0.04 [0.04-0.05]	0.04 [0.03-0.04]	0.04 [0.03-0.05]	0.05 [0.05-0.08]**
DHA, 22:6n-3	Center 1	0.6 [0.4-0.6]¶	0.5 [0.5-0.6]	0.4 [0.3-0.5]	0.2 [0.1-0.2]
	Center 2	0.3 [0.2-0.4]¶	0.5 [0.3-0.6]	0.4 [0.2-0.5]	0.3 [0.2-0.4]

Values are reported in units of wt % (g/100 g of fatty acids) and as median [IQR].
*Mother's milk: n = 19; donor milk: n = 7; center 1: n = 15; center 2: n = 11.
†Mother's milk: n = 21; donor milk, n = 6, source unknown: n = 2; center 1: n = 15; center 2: n = 14.
‡Mother's milk: n = 15; donor milk, n = 7; center 1: n = 9; center 2: n = 13.
§Mother's milk: n = 9; donor milk n = 1; center 1: n = 5; center 2: n = 5.
¶$P < .01$ comparing values between centers using the Wilcoxon rank-sum test.
**$P < .05$ comparing values between centers using the Wilcoxon rank-sum test.