HHS Public Access

Author manuscript

Lipids. Author manuscript; available in PMC 2017 April 01.

Published in final edited form as:

Lipids. 2016 April ; 51(4): 423–433. doi:10.1007/s11745-016-4130-4.

Daily Enteral DHA Supplementation Alleviates Deficiency in Premature Infants

Michelle L Baack, MD[1,2,3,*], **Susan E Puumala, PhD**[1,2], **Stephen E Messier, MD**[1,3], **Deborah K Pritchett, PharmD**[3], and **William S Harris, PhD**[1,2,4]

[1]Sanford Research, Children's Health Research Center, 2301 E. 60th Street North, Sioux Falls, SD, 57104, United States

[2]Sanford School of Medicine - University of South Dakota, 1400 W. 22nd St., Sioux Falls, SD, 57105, United States

[3]Boekelheide Neonatal Intensive Care Unit, Sanford Children's Hospital, 1600 W. 22nd St., PO Box 5039, Sioux Falls, SD, United States

[4]OmegaQuant Analytics, LLC, 5009 W. 12th St, Ste 8, Sioux Falls, SD, 57106, United States

Abstract

Docosahexaenoic acid (DHA) is an essential fatty acid (FA) important for health and neurodevelopment. Premature infants are at risk of DHA deficiency and circulating levels directly correlate with health outcomes. Most supplementation strategies have focused on increasing DHA content in mother's milk or infant formula. However, extremely premature infants may not reach full feedings for weeks and commercially available parenteral lipid emulsions do not contain preformed DHA, so blood levels decline rapidly after birth. Our objective was to develop a DHA supplementation strategy to overcome these barriers. This double-blind, randomized, controlled trial determined feasibility, tolerability and efficacy of daily enteral DHA supplementation (50mg/d) in addition to standard nutrition for preterm infants (24–34 weeks GA) beginning in the first week of life. Blood FA levels were analyzed at baseline, full feedings and near discharge in DHA (n=31) or placebo supplemented (n=29) preterm infants. Term peers (n=30) were analyzed for comparison. Preterm infants had lower baseline DHA levels ($p<0.0001$). Those receiving DHA had a progressive increase in circulating DHA over time (from 3.33% to 4.09%, $p<0.0001$) while placebo-supplemented infants (receiving standard neonatal nutrition) had no increase over time (from 3.35% to 3.32%). Although levels increased with additional DHA supplementation, preterm infants still had lower blood DHA levels than term peers (4.97%) at discharge ($p=0.0002$). No differences in adverse events were observed between the groups. Overall, daily enteral DHA supplementation is feasible and alleviates deficiency in premature infants.

Corresponding Author. Michelle Baack, MD; Children's Health Research Center, Sanford Research, 2301 60th Street N, Sioux Falls, SD 57104; Phone: 605-312-6420; Fax: 605-312-6971; michelle.baack@sanfordhealth.org.

Conflict of Interests:

Dr. William S. Harris has no foreseen financial gain from publication of this work. Additional authors declare no conflict of interest.

Keywords

Neonatal nutrition; premature infants; docosahexaenoic acid (DHA); long chain polyunsaturated fatty acids (LCPUFA); essential dietary lipids

Introduction

Long chain polyunsaturated fatty acids (LCPUFA), including docosahexaenoic acid (DHA) and arachidonic acid (ARA) are important for normal health and neurodevelopment. Because these essential fatty acids (FA) cannot sufficiently be synthesized *de novo* in fetus and preterm infant, a maternal source is necessary. LCPUFA are increasingly transferred from mother to fetus late in pregnancy, reaching peak accretion rates between 42 – 75 mg/d at 35 – 40 weeks of gestational age (GA) [1–4]. Premature infants, born before this process is complete, are at risk of deficiency [3]. Because DHA and ARA levels directly correlate with GA at birth [5], babies who are born the earliest are at the highest risk for deficiency [6]. Although there is increasing evidence to support the need for DHA supplementation in preterm infants, the optimal dose and method of administration are still unknown [3].

Previous studies demonstrate that circulating DHA levels are a marker of deficiency and that premature infants with higher circulating DHA levels have a lower risk of disease including necrotizing enterocolitis (NEC) [7], retinopathy of prematurity (ROP) [8] and bronchopulmonary dysplasia (BPD) [9, 10] as well as improved vision and neurodevelopmental outcomes [3, 11]. Benefits of increasing DHA provision appear promising, but are not conclusive [12], in part due to a wide variability in study design and supplementation methods [3]. Positive health benefits are most consistently found in studies using “high dose” DHA supplementation that more closely mimic *in utero* accretion rates [3, 11]. Large-scale intervention studies are necessary to further define the clinical benefits of DHA supplementation in preterm infants, but before this can be done, a dosing method that is both safe and efficacious (increases DHA levels) must be better developed. The objective of this study was to determine the feasibility, tolerability and biochemical efficacy of additional daily enteral DHA oil supplementation for premature infants.

The current standard is to provide LCPUFA in enteral feedings. Both DHA and ARA are available in human milk and in some commercially available infant formula in the United States. However, very premature infants typically do not reach full enteral feedings for several weeks and standard parenteral lipid emulsions do not provide these preformed LCPUFA. Additionally, premature infants have fewer fat stores and a decreased ability to convert the precursor FA, linoleic acid (LNA) and α-linolenic acid (ALA) that are provided in parenteral lipid emulsions, to ARA and DHA respectively [5, 13]. Likely for these reasons, DHA levels in premature infants decline rapidly after birth [6, 9, 14]. Even after full enteral feedings are reached, the standard dietary provision of DHA was developed based on the average world-wide content found in human milk (0.32% of total milk FA) [15] which is intended to meet the daily needs of healthy term infants, but is not adequate to make up for the deficiency of prematurity [3, 6]. A more rational approach would be to provide additional DHA in an amount that replicates the *in utero* accretion rate specific for the

infant's GA. Although this "high dose" DHA appears to be more efficacious, formula with this content is not commercially available. Improving breast milk DHA content to these levels through maternal fish oil supplementation is feasible and has been implemented in at least one institution [11, 14]; however breast milk levels are variable and dependent on maternal compliance [14] and not all infants in the Neonatal Intensive Care Unit (NICU) receive their mother's milk. Regardless, providing additional DHA in formula or breast milk remains dependent on the infant's ability to tolerate full volume enteral feedings which is variable between NICUs and often dependent on GA, size and clinical status.

To overcome these barriers, we carried out this prospective, single-center, double-blinded, randomized, placebo-controlled trial to investigate the feasibility, tolerability and efficacy of daily enteral DHA oil supplementation (50 mg/d) used from the first week of life to term GA or hospital discharge with a goal of alleviating DHA deficiency in premature infants.

Experimental Section

Study Cohort

The study included 90 infants (60 preterm and 30 term) who were admitted to the Boekelheide NICU at Sanford Health in Sioux Falls, SD between October 2012 and March 2014. Infants were less than or equal to seven days of age at enrollment. All participants (including those in the term reference group) were admitted to the NICU where staff were educated about the study details and had easy access to study team members and antioxidant treated spot cards for blood collection. Eligible preterm infants (24–34 weeks GA at birth) were further stratified into two groups: early preterm (GA of 24 0/7 to 28 6/7 weeks at birth) and late preterm (GA 29 0/7 to 33 6/7 weeks at birth). Adaptive enrollment was used to assure that extremely preterm infants were enrolled over the same time period as the more commonly admitted late preterm and term (≥38 weeks GA) infants. Detailed enrollment methods, inclusion/exclusion criteria, and detailed characteristics of the study population have been previously described [5].

Clinical Data

The following clinical data were collected from electronic medical records: maternal age, gravidity, race, diabetes status (problem list or glucose tolerance test), mode of delivery, and infant estimated GA, sex, reported race, birth weight, height, head circumference and growth chart percentile using the Fenton Growth Curve for preterm infants and the WHO Growth Chart for term infants [16, 17].

Ethics

The study was registered at ClinicalTrials.gov (NCT01908907) and was conducted under approval of the Sanford Health Institutional Review Board and with oversight from an independent Data Safety and Monitoring Board (DSMB). Recruitment, intervention and data collection, occurred only after written informed consent and HIPPAA authorization by parents who were required to be English speaking and over 18 years of age. All study team members were trained and certified to conduct human research including working with

vulnerable populations such as neonates. Infants deemed non-viable or clinically too unstable by the medical team were excluded or withdrawn from the study.

Intervention

Premature infants were randomized within 24 hours of enrollment to receive either 50mg/day (0.18ml) of DHA liquid (Pure Encapsulations®, Sudberg MA) (n=31) or placebo (Medium Chain Triglyceride - MCT® oil, Nestle Health Science, Florham Park, NJ) (n=29). Study oil administration began the day after randomization (during the first week of life) until discharge or 37 6/7 weeks GA, whichever came first. The DHA oil selected for this study provided 280mg/mL of DHA from an algal source (Life's DHA, Martek Biosciences Corporation, Columbia, MD) as used in most U.S. infant formulas. This formulation was selected to avoid inadvertent administration of contaminants (e.g. mercury, organic pollutants) potentially found in fish oil sources. Additionally, the algal source DHA oil is free of eicosapentaenoic acid (EPA), Vitamin D or A to avoid confounding factors in outcome analyses. Because the dose was not dependent upon the infant's ability to tolerate enteral feedings, daily provision began after enrollment in the first week of age unless the infant was deemed too unstable by the medical team. This dosing technique (mg/d rather than mg/kg/d) was selected to provide a uniform daily dose with the goal to replicate *in utero* DHA accretion rate of 42 to 75 mg/d [1–4] in the last trimester rather than using a weight based dose. In addition, as the infant grew it would be consuming additional DHA from standard feedings (breast milk or formula). Even with an anticipated enteral bioavailability of only 85%, the selected dose (50mg/d) should meet this goal, especially when given in addition to standard feedings which provide 3 – 23 mg/kg/d [3]. Study oil was dispensed through the Sanford Children's Hospital pharmacy in blinded fashion via amber colored syringes for daily administration at 1100 AM. If the infant had a gavage tube in place, oil was administered very slowly by gentle push through the feeding tube followed by a flush with 0.5 mL of sterile water to assure that most of the oil went through the tube (this was tested *in vitro* with good recovery). If the infant was feeding orally, oil was administered by mouth with a scheduled feeding. To decrease the possibility of absorption variability, no other medications were given within one hour of the study oil. Doses could be temporarily held by the medical team for clinical concerns or NPO status. The term reference group (n=30) had FA levels analyzed for comparison but did not receive any intervention.

Fatty Acid Analysis

Following enrollment, one drop of scavenged whole blood was collected on antioxidant pretreated filter paper at baseline for all infants as previously described [5]. Preterm infants also had whole blood collected after reaching full enteral feedings (at least 100kcal/kg/day or more) and again at term (≥38 weeks GA) or prior to discharge, whichever came first. Per study protocol, blood was collected only in conjunction with other laboratory testing to avoid additional needle sticks or central line access. Only 1–2 drops was collected on blood spot cards due to the high risk of anemia in this vulnerable population. Samples were analyzed for fatty acids from C:14 to C:24 by direct transesterification and gas chromatography at OmegaQuant Analytics (Sioux Falls, SD) using a validated method as previously described [5]. Blood LNA, ALA, ARA and DHA levels (i.e., composition) were expressed as a mol % of total blood FA recovered. Whole blood concentrations (μg/ml) were

also measured using an internal standard (22:3n3) as previously described [5] to account for potential changes in FA composition due to administration of intravenous lipid emulsions. Because the conclusions were not different, only composition data are reported here.

Outcome measures

This study was designed to determine feasibility, tolerability and dosing efficacy of enteral DHA supplementation but was not intended or powered to determine the effects of DHA on health related outcomes. Tolerability was measured by days to reach full enteral feedings (at least 100kcal/kg/day or more), days on study oil, GA at completion of the study and postnatal growth. Growth velocity was determined using weights (g), head circumference (cm) and length (cm) as recorded by the nursing staff. Weights were measured daily. Initially after birth weight was measured using the integral scale within the Ohmeda Giraffe OmniBed (Ohmeda Medical, Laurel, MD) according to the manufacturer's instructions. After the infant transitioned to an open crib, daily weights were measured using an Olympic Medical Smart Scale (Model 57361 - Olympic Medical, Seattle, WA). Length was measured (in triplicate) weekly using the Preemie Stadiometer (Ellard Instrumentation, Monroe, WA) for premature infants and the Newborn Stadiometer (Ellard Instrumentation) for term infants. Head circumference was also measured weekly using a Seca measuring band. Growth parameters were converted to growth percentiles using a Fenton growth curve for premature infants and the WHO growth chart for term infants. Adverse events were monitored throughout the study by the clinical team and an independent DSMB who met quarterly during the study period. Specific events of interest included thrombocytopenia, disseminated intravascular coagulopathy, intraventricular hemorrhage, periventricular leukomalacia, BPD, ROP, NEC, sepsis, milk soy protein intolerance, gastroesophageal reflux disease and death. Whole blood composition of LNA, ALA, ARA and DHA were compared between treatment groups and to the term reference group at baseline and discharge.

Statistical Analysis

Descriptive statistics were calculated for all variables and are expressed as means and standard deviations for continuous variables and frequencies and percents for categorical variables. All analyses used the intent-to-treat study population. Adverse events of specific interest were analyzed using Fisher's exact test. Linear mixed models were used to explore growth over time (weight, length and head circumference). These models included a random effect for intercepts and slopes to account for subject specific growth over time as well as a random effect for possible correlation between twins and triplets present in the data set. Each random effect was tested for inclusion in the model using the method suggested by Verbeke and Molenberghs [18]. Using this approach it was determined that the random effect for multiple births was not needed, so this was removed from the analysis. Fixed effects initially included both a linear and quadratic time effect, GA at birth, treatment group, varying indicator for time to reach full feeds, and interactions with the linear and quadratic time effect for all other variables. A backwards selection model was used to eliminate non-significant interactions.

A linear mixed model was used to assess possible differences in time to full feeds, days on study drug, and gestational age at discharge. Time to full feeds was transformed using natural logarithmic transformation. These models included a random effect for multiples, which was maintained in the model after testing. Fixed effects included treatment and GA group for time to full feeds and days on study drug and treatment effect for gestational age at discharge. Sensitivity analysis was also performed excluding those with intrauterine growth restriction (IUGR).

Linear mixed models were also used to examine the association between each FA of interest and treatment group over time. Since only three time points were available for FA measurement, only a random intercept was included in the models. For these models, the random effect for multiples was again found to be not needed and removed. Primary outcome variables for this analysis included LNA, ALA, ARA and DHA. Only early/late preterm status was included in the model as a covariate since this was a stratification variable. Continuous dependent variables were transformed using the natural logarithm as needed to meet the assumptions of the regression model (this included LNA and ALA). To account for doses held by the medical team, sensitivity analysis was performed for all main outcome variables controlling for the number of days on study oil. This did not change any of the study results. Model diagnostics were examined to ensure that models were not overly influenced by outliers. Differences between treatment groups and term controls at discharge were assessed using an ANOVA model with t-tests for pairwise comparisons. Estimates were different in these models since we were not able to control for early/late preterm groups in this analysis.

Results

Study Demographics

Between October 2012 and March 2014, 140 preterm infants were found to be eligible for the study. As per protocol, 60 preterm and 30 term infants were enrolled. The most common reason premature infants were not enrolled was parental refusal and for term infants that infants did not require any clinically mandated laboratory testing or that the target enrollment for that period had already been met due to adaptive enrollment. Study demographics were previously reported [5] for all infants in the study which included 13 early-preterm infants (mean GA 26.6 weeks; range 25–28 weeks), 47 later-preterm infants (mean GA 31.6 weeks; range 29–33 weeks) and 30 term infants (mean GA 39.3 weeks; range 38–41).

Of the preterm infants, 29 were randomized to receive placebo and 31 to receive DHA. Baseline characteristics were compared between the treatment groups (Table 1). There were no significant differences in gender, ethnicity, race, diabetes exposure or multiple gestations. However, a significant difference in birth size was discovered. Infants randomized to receive DHA were significantly smaller than infants randomized to the placebo group. Mean length percentile was significantly lower; weight and head circumference percentiles trended this way as well. On further inspection, there were 4 (13%) IUGR infants in the DHA supplemented group and only 1 (3%) in the placebo supplemented group (IUGR infants had both length and weight $<10^{th}$ % by definition). This is important when interpreting group

data because IUGR may negatively affect baseline LCPUFA levels, postnatal feeding progression, growth and adverse event risk in the DHA supplemented group.

Feasibility and tolerability of daily enteral DHA administration

Staff reported no difficulty with administration by gavage tube or orally with feedings. Infants enrolled in the study tolerated daily enteral dosing of study oil well. There were no reports of intolerance, loose stools or other side effects noted in any enrolled patient by parents or the clinical care team. There were no significant differences in the number of infants with gastrointestinal problems during their NICU stay (gastroesophageal reflux, milk soy protein intolerance). The DHA supplemented infants had a non-significant increase in days to reach full feeds of about 2.6 days compared to the placebo group ($p = 0.07$). This difference was found to be similar after excluding the IUGR infants in both treatment groups. However, after adjusting for birth weight, the increase in days to reach full feedings was only 1.8 days more for DHA vs. placebo supplemented infants. To further evaluate this, the days on study oil (as an indicator of doses held by the medical team) and GA at study end (discharge or term GA) were also assessed. There were no significant group differences in the days on study oil (DHA group 34±19.12 days, placebo group 33.71±16.32 days; $p=0.84$) or the GA at study end (DHA group 36.67±1.06 weeks, range 34–39 weeks; placebo group 36.45 ±0.97 weeks, range 35–38 weeks; $p=0.35$).

There was no difference in the incidence of adverse events between DHA and placebo supplemented preterm infants. (Table 2). Importantly, there were no cases of NEC, intraventricular hemorrhage or bleeding disorders, and the rate of sepsis was not different between preterm groups. One death occurred during the study. This infant was enrolled in the DHA group, but due to an unstable clinical status never received study oil before dying of sepsis.

Growth

Daily enteral DHA supplementation did not impair growth (Figure 1). As expected, the linear mixed model implied that infants born at an earlier GA were smaller at birth and had slower growth prior to reaching full enteral feedings ($p\leq0.0001$) but thereafter the growth trajectory increased. The model indicated a significant group difference in size at birth with the infants randomized to receive DHA being smaller (weight, length and head circumference). Despite this, there were no undesirable differences in growth over time. In fact, DHA supplemented infants had an increased rate of linear growth compared to placebo supplemented infants ($p=0.04$) (Figure 1B).

LCPUFA levels

As previously reported, preterm infants had significantly lower DHA and ARA levels at baseline than their term peers ($p<0.0001$) and levels directly correlated with GA so that those born earliest were at highest risk of LCPUFA deficiency [5]. For DHA, (Figure 2-A), there were no baseline differences between the treatment groups, and DHA supplementation in premature infants significantly increased DHA levels over time ($p<0.0001$). Placebo supplemented preterm infants remained deficient with no increase in DHA above baseline levels, so that by discharge the DHA supplemented infants had significantly higher levels

than controls (p<0.001). Despite improvement, DHA levels at discharge were still significantly lower than those of term infants (p<0.001) (Table 3).

For ARA, (Figure 2-B), baseline levels were lower in infants randomized to receive DHA. These results were similar after excluding IUGR infants. This difference disappeared by the time full feedings were reached as the DHA supplemented infants had an increase in ARA levels (p=0.003). Both preterm groups had lower ARA levels than term infants at discharge (p<0.0001).

In contrast to DHA and ARA, their 18 carbon precursors (ALA and LNA) were significantly higher in preterm infants compared to term peers (p<0.0001). Both ALA and LNA were highest at baseline, presumably due to infusion of parenteral lipid emulsion containing a relatively high level of these precursors FA but no preformed DHA or ARA. (Figure 2–C and D) For (ALA), there was no difference in mean levels by treatment group, but over time the baseline levels decreased in premature infants, specifically from baseline to full feeds (p<0.001). Even so, by discharge, preterm infants still had significantly higher mean ALA levels than term infants (p<0.001) (Figure 2–C). LNA levels were higher in the DHA supplemented preterm group at baseline (p = 0.005), but not at any other time point. LNA levels decreased over time in this group, specifically from baseline to full feeds (p<0.0001). Placebo supplemented preterm infants had a significant decrease in LNA from baseline to full feeds, but then a similar increase from full feeds to discharge. Both preterm groups have significantly higher LNA levels at discharge compared to their term peers (*p<0.0001).

The DHA supplemented group had higher LA levels at baseline, but there was no difference between groups at any other time-point (Figure 2-D). ALA and LNA levels both remained higher than those of term infants at discharge.

Discussion

It is well recognized that LCPUFA are essential for normal health and development and that higher DHA levels are directly associated with improved outcomes in premature infants [3, 9] yet, the optimal method of administration and amount necessary to obtain benefit remains unclear. This single center, randomized, placebo-controlled trial demonstrates that daily enteral DHA supplementation (50mg/d) during the NICU stay is feasible, well tolerated and largely alleviates DHA deficiency. Our findings highlight the fact that providing preterm infants with DHA via mother's milk or formula using the current standard of care (as in our placebo group) does not increase blood DHA levels throughout the NICU stay. Giving premature infants additional daily enteral DHA (starting before full enteral feedings are reached and at a dose that more closely approximates *in utero* accretion rates) significantly increases blood DHA levels. These findings set the stage for larger trials aimed at improving clinical outcomes for high-risk infants using daily enteral DHA supplementation.

The optimal way to provide DHA for premature infants is difficult to determine from the current literature. This is due to marked variation among studies (study population, administration methods, doses and even outcome parameters). Most DHA supplementation studies have aimed to improve neurodevelopmental outcomes. Because breastfed infants had

a higher IQ [19] and outcomes correlated to LCPUFA fat content [20], the initial approach was to add DHA to formula at a comparable amount found in human milk. Following this concept, many studies have administered DHA through breast milk (via maternal supplementation) [21–25] or formula at various doses (from 0.14 % to over 1.0 % of FA as DHA) [10, 26–30]. However, supplementation by these methods is dependent on the infant's ability to tolerate full volume enteral feedings which occurs at variable rates and often not for several weeks in extremely premature infants. Infants that are NPO or receiving incrementally increasing feedings rely on intravenous nutritional support for extended periods. Standard parenteral soy-bean based lipid emulsions provide essential FA as LNA and ALA, but do not contain any preformed DHA or ARA. Conversion of these precursor FAs to LCPUFAs is limited *in vivo*, especially in premature infants [2, 5, 6]. Although there are parenteral lipid emulsions with preformed LCPUFAs (Omegaven® and SMOF®), these are not approved for routine use in infants and are not commercially available in the United States.

Likely due to an early disruption in maternal-fetal FA transport, decreased fat stores, increased demands and poor provision early in life, DHA levels in preterm infants decline rapidly after birth [6, 9]. This cannot be prevented by supplementation through feedings alone. Moreover, some of the proposed benefits of DHA supplementation (decreased incidence of NEC, BPD and ROP) may be dependent on LCPUFA status during this early time-point. After birth the premature infant transitions to postnatal life too soon and is precipitously exposed to oxidative stress and inflammation that interrupts normal organ growth and development [31]. This oxidative stress can be exacerbated by the administration of oxygen and parenteral nutrition [32]. LCPUFAs play a key protective role through anti-oxidant and anti-inflammatory mechanisms [33–35]. Conceivably, this is why DHA supplementation studies with variable timing have reported mixed results. Specifically, parenteral administration of omega-3 containing lipid emulsion early in life (the first 7–14 days) was associated with a lower incidence and severity of ROP [8, 36]. This is encouraging and suggests that early supplementation may have additional protective benefits, but even though parenteral administration improved DHA status early after birth blood levels returned to baseline after discontinuation (by 4 weeks of age) [8, 36, 37]. So, although this method is associated with a decreased incidence of ROP [8, 36], it is unknown if this will improve longer term neurodevelopmental outcomes as well.

In an attempt to deliver a more consistent daily provision of DHA early after birth without concerns of feeding advancement and intravenous access, our study investigated daily enteral administration of DHA oil (from the first week of life to discharge). We found that daily enteral DHA dosing is feasible, well tolerated and improves the DHA status of premature infants. After starting supplementation, DHA levels did not fall below baseline (average of 6.8 days of life in our study) but rather rose over time. This administration method provides promise when the goal is to improve both short- and long-term neonatal outcomes in premature infants through improved DHA status from birth on.

This study demonstrates that daily enteral DHA supplementation is feasible, but the optimal daily dose for premature infants must still be determined. Given our findings and those of others [14], we estimate that 50–100mg/day administered early after birth will correct the

DHA deficit found in premature infants. We show that 50mg/day of DHA prevented a decline in blood levels and once full enteral feedings were also reached DHA levels steadily increased in supplemented preterm infants but not controls. A higher dose is likely necessary to bring DHA levels up to those of term GA infants. In our study, DHA levels rose from 2.88 mol% (or 3.33 wt %) at baseline (6 days) to 3.55 mol% (4.09 wt%) near discharge, but did not quite reach the mean level of 4.31 mol% (4.97 wt%) found in the term reference group. However, one caveat of the study was that the average GA at study end (typically discharge) for the preterm supplemented group was 36.8 weeks and the average GA of the term reference group was 39.3 weeks. So it is possible that if DHA was continued longer and levels were measured at the same GA time-point that the supplemented preterm infants would have DHA levels similar to that of term infants.

A recent study by Collins, et al. compared 40, 80 and 120mg/kg/day of DHA (in tuna oil) given to premature infants in the NICU for the first 28 days [14]. Both our study and theirs enrolled infants with similar characteristics (mean GA of 30 vs. 29 weeks), started enteral dosing in the first week of life and gave daily enteral DHA doses in addition to feedings with the standard DHA content. Differences between our study and that from Collins, et al. include the omega-3 oil source (algal source DHA oil vs. tuna oil with additional additives such as EPA and fat soluble vitamins), length of treatment (until discharge vs. 28 days) and baseline DHA levels (higher in the Australian cohort, likely due to regional dietary and supplementing differences). It is difficult to directly compare efficacy based on FA levels alone because it is the standard practice for some lactating mothers in Australia to take omega-3 supplements. Additionally infants used in their reference group were from a previous study using high-dose maternal supplementation (DHA for the Improvement of Neurodevelopmental Outcomes - DINO trial) [29] and thus had a higher DHA level (5.09 wt %) than our term reference group (4.31 mol% or 4.97 wt %) [14]. This would be anticipated given regional, dietary and supplementation differences between these two study sites, but it should also be noted that the term infants in our study were admitted to the NICU which suggests that they may not necessarily be “healthy” infants. Overall, the combined studies suggest that daily enteral doses of 50–100 mg of DHA/d in addition to the standard provision in feedings will significantly alleviate deficiency in this high risk population.

Importantly, both studies added to a growing body of literature that daily enteral DHA supplementation is well tolerated at a wide range of doses [14, 38]. Although nutritional supplements are typically not evaluated by the Food and Drug Administration, the agency has confirmed the general safety of fish/DHA oil with no significant adverse effects being described in humans at doses ranging from 25 to 5900mg/kg/day [39]. This safety extends to the infant population, including premature infants. Henriksen, et al. supplemented premature infants with 32mg/day of DHA with no adverse events and improved neurodevelopmental outcomes [38]. In a similar population, the DINO trial utilized 1.0 % of total FA, equivalent to 50mg/kg/day, in formula or breast milk (through maternal supplementation) with no growth impairment or other adverse reactions [11]. Several other studies have investigated *fish oil* in very high doses (1g/kg/day providing 120–375mg/day of DHA) for treatment of Parenteral Nutrition Associated Liver Disease [40–43]. Two patients in this study had bleeding, but also had significant liver disease as the presumed cause [44]. However, because

of the effect of omega-3 FA on platelet aggregation [45] this remains an adverse event that should be carefully tracked in future studies with premature infants.

Interestingly, adverse events in our study group were overall very low for the population of interest. It is possible that selection bias played a role due to parents consenting only if their baby was “off to a good start”. Nonetheless, infants receiving daily enteral DHA, tolerated the dosing well with no increased adverse events compared to controls. There was a non-significant increase in time to reach full feedings, but there was no difference in the days on DHA (representing doses held by the clinical team due to NPO status) or length to study end (usually at discharge). Despite past concerns that DHA supplementation without additional ARA could be associated with poor growth [46], premature infants that received DHA had better length growth compared to controls. It should be noted that premature infants in both groups remained deficient in ARA and had significantly higher ALA and LNA levels compared to term infants at discharge. Although this was not different between DHA and placebo supplemented preterm infants, this suggests that additional nutritional lipid provisions may need closer scrutiny. Caution should be taken that all essential FA levels are tracked in any future LCPUFA supplementation studies.

This study demonstrates the feasibility, tolerability and efficacy of daily enteral DHA supplementation for premature infants. Our results should aid in the development of larger multicenter studies aimed at improving both short- and long-term outcomes in premature infants through optimization of daily enteral DHA provision. Defining goals for circulating FA levels at specific, clinically relevant times would be helpful; and ongoing monitoring of feeding advancement, adverse events and effects on other essential FA levels (ARA, LNA, ALA) is necessary. We anticipate this method of DHA supplementation will consistently increase circulating DHA levels and thereby improve clinical outcomes for very premature infants.

Acknowledgments

We gratefully acknowledges our exceptional study team (Von “Mimi” Chau, Diane Hahn, Katherine Burgess, Dawn Hansen, Jessica Howard, Carmen Sandman, Melissa Hemenway), DSMB members (Pam Koepsell, Dr. Lisa Jaton, Dr. Paul Thompson, Dr. Dennis Stevens, Michele Sturdevant, and Dr. Amanda Jensen), NICU staff nurses, pharmacy staff (Linda Oyen), neonatologists, and especially our NICU babies and their families—it is such a privilege to care for you. Funding for this study was provided by a Sanford Health Seed Grant, a Gerber Foundation Pediatric Nutrition Grant (PN12-005-1372-3069), and Sanford Research. Dr. Baack also receives support from the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health (K08HD078504) and an NIH Center of Biomedical Research Excellence (COBRE) grant (P20 GM103620-01A1). Further information about this trial and ongoing efforts may be found at ClinicalTrials.gov (NCT01908907).

Dr. William S. Harris who serves as a senior mentor to Dr. Baack, is the Founder and President of OmegaQuant, LLC. Funding sponsors had no role in study design, implementation, data collection, analyses, interpretation or publication.

Abbreviations

ALA	α-linolenic acid
ARA	arachidonic acid

BPD	bronchopulmonary dysplasia
DSMB	Data Safety and Monitoring Board
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FA	fatty acids
GA	gestational age
IUGR	intrauterine growth restriction
LNA	linoleic acid
LCPUFA	long chain polyunsaturated fatty acids
NEC	necrotizing enterocolitis
NICU	Neonatal Intensive Care Unit
ROP	retinopathy of prematurity

References

1. Kuipers RS, Luxwolda MF, Offringa PJ, Boersma ER, Dijck-Brouwer DA, Muskiet FA. Fetal intrauterine whole body linoleic, arachidonic and docosahexaenoic acid contents and accretion rates. Prostaglandins Leukot Essent Fatty Acids. 2012; 86:13–20. [PubMed: 22115845]
2. Innis SM. Essential fatty acid transfer and fetal development. Placenta. 2005; 26(Suppl A):S70–S75. [PubMed: 15837071]
3. Harris WS, Baack ML. Beyond building better brains: bridging the docosahexaenoic acid (DHA) gap of prematurity. J Perinatol. 2015; 35:1–7. [PubMed: 25357095]
4. Lapillonne AJC. Reevaluation of the DHA requirement for the premature infant. Prostaglandins Leukot Essent Fatty Acids. 2009:143–150. [PubMed: 19577914]
5. Baack ML, Puumala SE, Messier SE, Pritchett DK, Harris WS. What is the relationship between gestational age and docosahexaenoic acid (DHA) and arachidonic acid (ARA) levels? Prostaglandins Leukot Essent Fatty Acids. 2015; 100:5–11. [PubMed: 26205427]
6. Lapillonne, AEdTS.; Kermorvant-Duchemin, E. Postnatal docosahexaenoic acid deficiency is an inevitable consequence of current recommendations and practice in preterm infants. Neonatology. 2010; 98:397–403. [PubMed: 21051909]
7. Carlson SE, Montalto MB, Ponder DL, Werkman SH, Korones SB. Lower incidence of necrotizing enterocolitis in infants fed a preterm formula with egg phospholipids. Pediatr Res. 1998; 44:491–498. [PubMed: 9773836]
8. Pawlik D, Lauterbach R, Walczak M, Hurkala J, Sherman MP. Fish-oil fat emulsion supplementation reduces the risk of retinopathy in very low birth weight infants: a prospective, randomized study. JPEN J Parenter Enteral Nutr. 2014; 38:711–716. [PubMed: 23963690]
9. Martin CR, Dasilva DA, Cluette-Brown JE, Dimonda C, Hamill A, Bhutta AQ, Coronel E, Wilschanski M, Stephens AJ, Driscoll DF, et al. Decreased postnatal docosahexaenoic and arachidonic acid blood levels in premature infants are associated with neonatal morbidities. J Pediatr. 2011; 159:743–749. e741–e742. [PubMed: 21658712]
10. Manley BJ, Makrides M, Collins CT, McPhee AJ, Gibson RA, Ryan P, Sullivan TR, Davis PG. High-dose docosahexaenoic acid supplementation of preterm infants: respiratory and allergy outcomes. Pediatrics. 2011; 128:e71–e77. [PubMed: 21708809]
11. Makrides M, Gibson RA, McPhee AJ, Collins CT, Davis PG, Doyle LW, Simmer K, Colditz PB, Morris S, Smithers LG, et al. Neurodevelopmental outcomes of preterm infants fed high-dose

docosahexaenoic acid: a randomized controlled trial. JAMA. 2009; 301:175–182. [PubMed: 19141765]

12. Zhang P, Lavoie PM, Lacaze-Masmonteil T, Rhainds M, Marc I. Omega-3 long-chain polyunsaturated fatty acids for extremely preterm infants: a systematic review. Pediatrics. 2014; 134:120–134. [PubMed: 24913791]
13. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. Curr Opin Clin Nutr Metab Care. 2002; 5:127–132. [PubMed: 11844977]
14. Collins CT, Sullivan TR, McPhee AJ, Stark MJ, Makrides M, Gibson RA. A dose response randomised controlled trial of docosahexaenoic acid (DHA) in preterm infants. Prostaglandins Leukot Essent Fatty Acids. 2015; 99:1–6. [PubMed: 25997653]
15. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007; 85:1457–1464. [PubMed: 17556680]
16. Fenton TR, Nasser R, Eliasziw M, Kim JH, Bilan D, Sauve R. Validating the weight gain of preterm infants between the reference growth curve of the fetus and the term infant. BMC Pediatr. 2013; 13:92. [PubMed: 23758808]
17. Group WMGRS. WHO Child Growth Standards: Growth velocity based on weight, length and head circumference: Methods and development. 2009:242.
18. G, V.; G, M. Linear Mixed Models for Longitudinal Data. New York: Springer; 2000.
19. Lucas A, Morley R, Cole TJ, Lister G, Leeson-Payne C. Breast milk and subsequent intelligence quotient in children born preterm. Lancet. 1992; 339:261–264. [PubMed: 1346280]
20. Agostoni C, Marangoni F, Lammardo AM, Giovannini M, Riva E, Galli C. Breastfeeding duration, milk fat composition and developmental indices at 1 year of life among breastfed infants. Prostaglandins Leukot Essent Fatty Acids. 2001; 64:105–109. [PubMed: 11237477]
21. Jensen CL, Voigt RG, Prager TC, Zou YL, Fraley JK, Rozelle JC, Turcich MR, Llorente AM, Anderson RE, Heird WC. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. Am J Clin Nutr. 2005; 82:125–132. [PubMed: 16002810]
22. Lauritzen L, Jorgensen MH, Mikkelsen TB, Skovgaard lM, Straarup EM, Olsen SF, Hoy CE, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes. Lipids. 2004; 39:195–206. [PubMed: 15233397]
23. Gibson RA, Neumann MA, Makrides M. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. Eur J Clin Nutr. 1997; 51:578–584. [PubMed: 9306083]
24. Lauritzen L, Jorgensen MH, Olsen SF, Straarup EM, Michaelsen KF. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. Reprod Nutr Dev. 2005; 45:535–547. [PubMed: 16188206]
25. Makrides M, Neumann MA, Gibson RA. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast milk composition. Eur J Clin Nutr. 1996; 50:352–357. [PubMed: 8793415]
26. Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, Erickson JR, Fitzgerald KM, Dobson V, Innis SM, et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. Pediatrics. 2001; 108:372–381. [PubMed: 11483802]
27. Birch EE, Garfield S, Castaneda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. Early Hum Dev. 2007; 83:279–284. [PubMed: 17240089]
28. Innis SM, Adamkin DH, Hall RT, Kalhan SC, Lair C, Lim M, Stevens DC, Twist PF, Diersen-Schade DA, Harris CL, et al. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. J Pediatr. 2002; 140:547–554. [PubMed: 12032520]
29. Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of two doses of docosahexaenoic acid (DHA) in the diet of preterm infants on infant fatty acid status: results from the DINO trial. Prostaglandins Leukot Essent Fatty Acids. 2008; 79:141–146. [PubMed: 18951004]

30. Vanderhoof J, Gross S, Hegyi T, Clandinin T, Porcelli P, DeCristofaro J, Rhodes T, Tsang R, Shattuck K, Cowett R, et al. Evaluation of a long-chain polyunsaturated fatty acid supplemented formula on growth, tolerance, and plasma lipids in preterm infants up to 48 weeks postconceptional age. J Pediatr Gastroenterol Nutr. 1999; 29:318–326. [PubMed: 10467999]
31. Marseglia L, D'Angelo G, Manti S, Arrigo T, Barberi I, Reiter RJ, Gitto E. Oxidative stress-mediated aging during the fetal and perinatal periods. Oxid Med Cell Longev. 2014; 2014:358–375.
32. Mohamed I, Elremaly W, Rouleau T, Lavoie JC. Oxygen and parenteral nutrition two main oxidants for extremely preterm infants: 'It all adds up'. J Neonatal Perinatal Med. 2015; 8:189–197. [PubMed: 26485550]
33. Bazan NG, Molina MF, Gordon WC. Docosahexaenoic Acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Annu Rev Nutr. 2011; 31:321–351. [PubMed: 21756134]
34. Liu Q, Wu D, Ni N, Ren H, Luo C, He C, Kang JX, Wan JB, Su H. Omega-3 polyunsaturated fatty acids protect neural progenitor cells against oxidative injury. Mar Drugs. 2014; 12:2341–2356. [PubMed: 24786451]
35. Lu J, Jilling T, Li D, Caplan MS. Polyunsaturated fatty acid supplementation alters proinflammatory gene expression and reduces the incidence of necrotizing enterocolitis in a neonatal rat model. Pediatr Res. 2007; 61:427–432. [PubMed: 17515866]
36. Beken S, Dilli D, Fettah ND, Kabatas EU, Zenciroglu A, Okumus N. The influence of fish-oil lipid emulsions on retinopathy of prematurity in very low birth weight infants: a randomized controlled trial. Early Hum Dev. 2014; 90:27–31. [PubMed: 24314586]
37. Pawlik D, Lauterbach R, Walczak M, Hurkala J. Docosahexaenoic acid (DHA) concentration in very low birth weight newborns receiving a fish-oil based fat emulsion from the first day of life. Preliminary clinical observation. Med Wieku Rozwoj. 2011; 15:312–317. [PubMed: 22006486]
38. Henriksen C, Haugholt K, Lindgren M, Aurvag AK, Ronnestad A, Gronn M, Solberg R, Moen A, Nakstad B, Berge RK, et al. Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. Pediatrics. 2008; 121:1137–1145. [PubMed: 18519483]
39. Lien VW, Clandinin MT. Dietary assessment of arachidonic acid and docosahexaenoic acid intake in 4–7 year-old children. J Am Coll Nutr. 2009; 28:7–15. [PubMed: 19571154]
40. Tomsits E, Pataki M, Tolgyesi A, Fekete G, Rischak K, Szollar L. Safety and efficacy of a lipid emulsion containing a mixture of soybean oil, and medium-chain triglycerides, olive oil, and fish oil: a randomised, double-blind clinical trial in premature infants requiring parenteral nutrition. J Pediatr Gastroenterol Nutr. 2010; 51:514–521. [PubMed: 20531018]
41. Lien EL. Toxicology and safety of DHA. Prostaglandins Leukot Essent Fatty Acids. 2009; 81:125–132. [PubMed: 19501496]
42. Puder M, Valim C, Meisel JA, Le HD, de Meijer VE, Robinson EM, Zhou J, Duggan C, Gura KM. Parenteral fish oil improves outcomes in patients with parenteral nutrition-associated liver injury. Ann Surg. 2009; 250:395–402. [PubMed: 19661785]
43. Rollins MD, Scaife ER, Jackson WD, Meyers RL, Mulroy CW, Book LS. Elimination of soybean lipid emulsion in parenteral nutrition and supplementation with enteral fish oil improve cholestasis in infants with short bowel syndrome. Nutr Clin Pract. 2010; 25:199–204. [PubMed: 20413701]
44. Tillman EM, Crill CM, Black DD, Hak EB, Lazar LF, Christensen ML, Huang EY, Helms RA. Enteral fish oil for treatment of parenteral nutrition-associated liver disease in six infants with short-bowel syndrome. Pharmacotherapy. 2011; 31:503–509. [PubMed: 21923431]
45. Phang M, Lincz LF, Garg ML. Eicosapentaenoic and docosahexaenoic acid supplementations reduce platelet aggregation and hemostatic markers differentially in men and women. J Nutr. 2013; 143:457–463. [PubMed: 23390192]
46. Carlson SE, Cooke RJ, Werkman SH, Tolley EA. First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. Lipids. 1992; 27:901–907. [PubMed: 1491608]

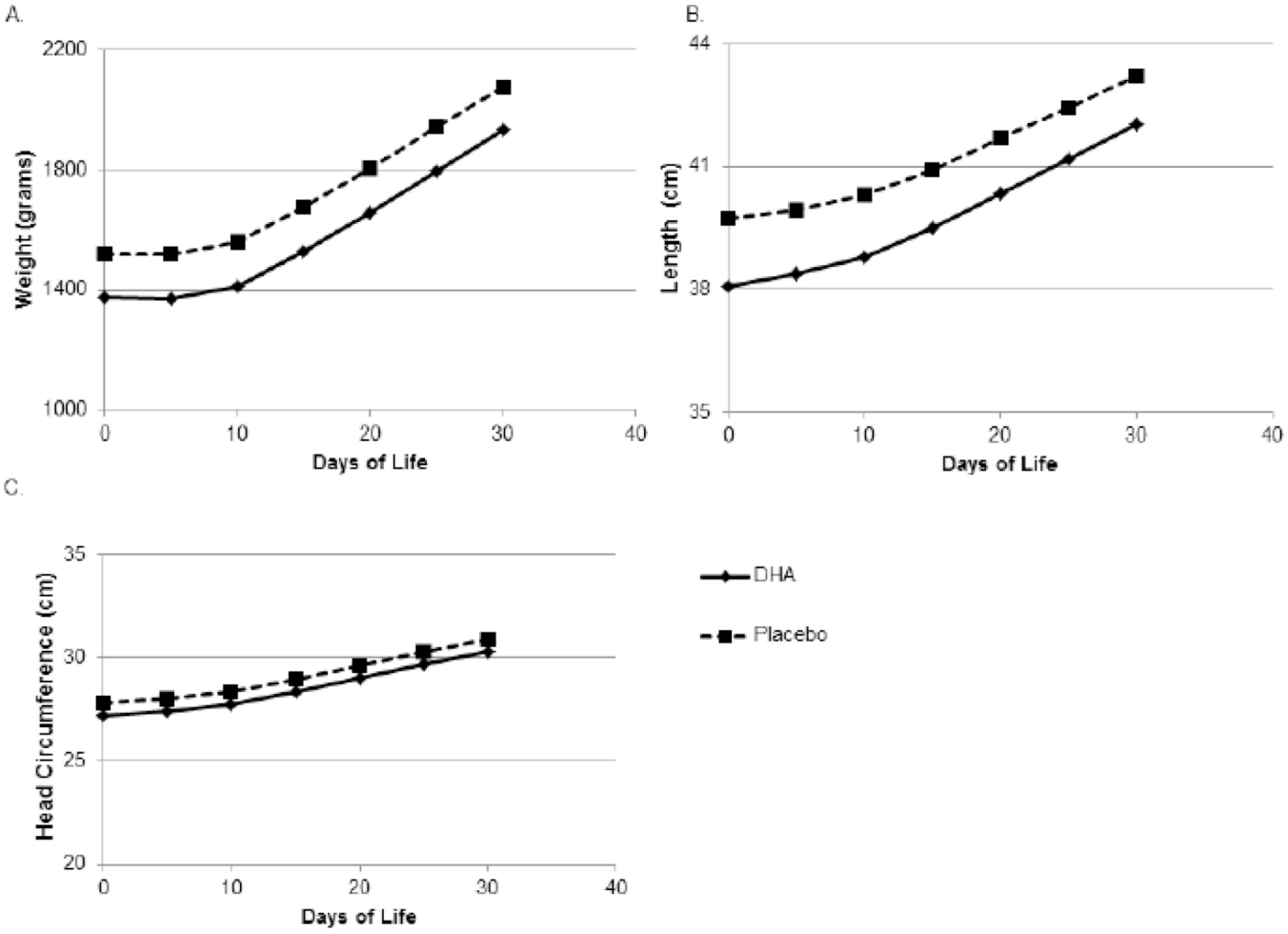

Fig. 1.

Postnatal growth model estimates for (A.) weight, (B.) length, and (C.) head circumference are represented graphically for infants born at the mean of 30 weeks GA. Dashed lines represent placebo controls and solid lines represent DHA supplemented infants. *Compared to controls, the DHA supplemented infants had increased length over time (p=0.04), otherwise, DHA supplementation did not affect postnatal growth.

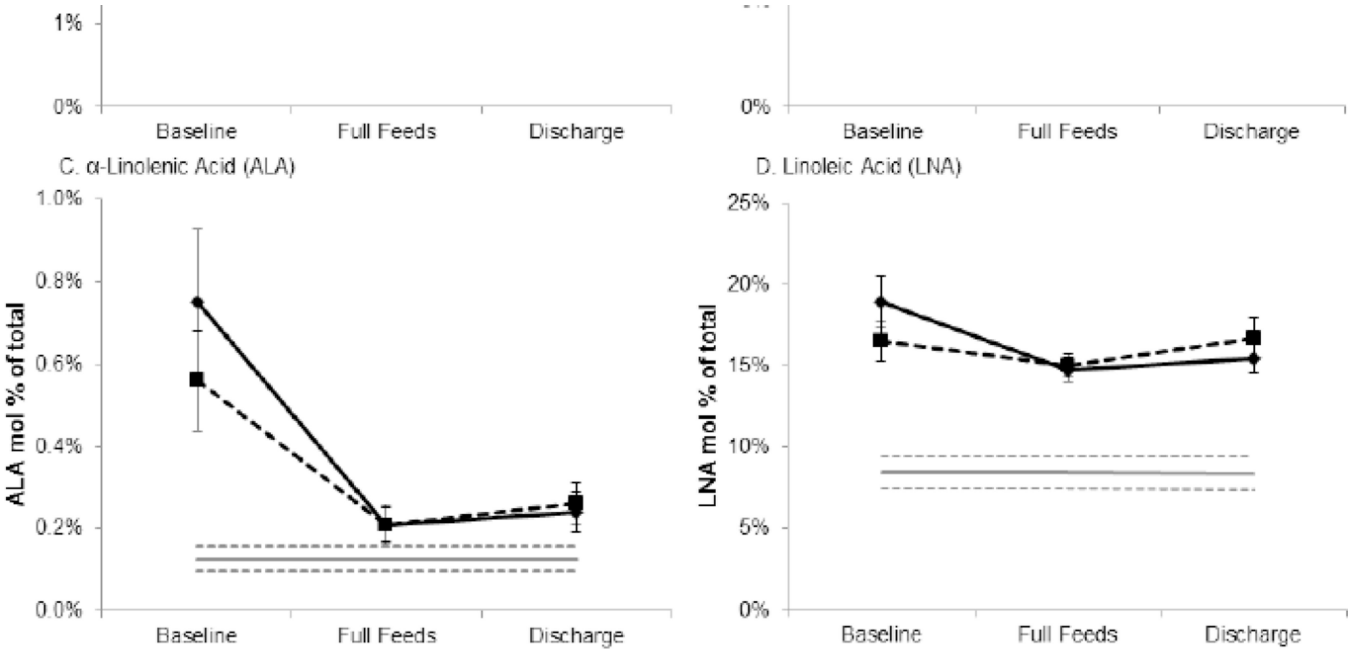

Fig. 2.

Fatty acid levels at baseline (first week), after reaching full feedings (100kcal/kg/d) and near discharge in placebo (dashed black line) and DHA supplemented (solid black line) preterm infants. Levels expressed as mol % with mean ± SD. For illustration only, a solid gray line represents the mean DHA level in the term reference group; the dashed gray line illustrates the 95% CI. †group comparison, $p<0.01$; *discharge level compared to that of term peers, $p<0.01$.

Table 1

Baseline demographics by treatment group

Variable	Category	Placebo (n=29)	DHA (n=31)	p-value
Gender	Male	14 (48.3%)	16 (51.6%)	0.99
	Female	15 (51.7%)	15 (48.4%)	
Ethnicity	Hispanic	0 (0.0%)	0 (0.0%)	NA
	Non-Hispanic	25 (86.2%)	28 (90.3)%	
	Unknown	4 (13.8%	3 (9.7%)	
Race	Caucasian	24 (82.8%)	29 (93.5%)	0.12
	Pacific Islander	1 (3.4%)	0 (0.0%)	
	Native American	4 (13.8%)	1 (3.2%)	
	Unknown	0 (0.0%)	1 (3.2%)	
Diabetic Mother	Yes	3 (10.3%)	2 (6.5%)	0.67
	No	26 (86.7%)	29 (93.5%)	
Type of Birth	Singleton	20 (69.0%)	14 (45.2%)	0.18
	Twins	7 (24.1%)	13 (41.9%)	
	Triplets	2 (6.9%)	4 (12.9%)	
Birth Weight *		51.34 (21.99)	39.74 (23.89)	0.06
Birth Length *		46.18 (27.58)	25.87 (21.89)	0.003
Head Circumference *		46.59 (25.71)	39.00 (25.37)	0.25
Maternal Age		29.79 (6.00)	29.84 (5.20)	0.98
Gestational Age		30.69 (2.42)	30.35 (2.46)	0.60

Docosahexaenoic acid, DHA

* Growth parameters reported as percentile on the Fenton Growth Curve at birth; one infant did not have length recorded

Categorical data represented as frequency (%) with comparison by Fisher's exact test

Continuous variables are expressed as mean (SD) and differences were analyzed by Student's t-test

Table 2

Adverse events by treatment group

Adverse Event	DHA (n=31)	Placebo (n=29)	p value
Necrotizing enterocolitis	0 (0.0%)	0 (0.0%)	NA
Disseminated intravascular coagulation	0 (0.0%)	0 (0.0%)	NA
Bronchopulmonary dysplasia	2 (6.5%)	3 (10.3%)	0.67
Sepsis	3 (9.7%)	3 (10.3%)	0.99
Retinopathy of prematurity	7 (22.6%)	5 (17.2%)	0.75
Gastroesophageal reflux disease	6 (19.4%)	4 (13.8%)	0.73
Milk soy protein intolerance	4 (12.9%)	4 (13.8%)	0.99
Intraventricular hemorrhage	0 (0.0%)	1 (3.4%)	0.48
Periventricular leukomalacia	0 (0.0%)	1 (3.4%)	0.48
Thrombycytopenia	1 (3.2%)	0 (0.0%)	0.99
Death*	1 (3.2%)	0 (0.0%)	0.99

Categorical data represented as frequency (%); Comparison by Fisher's exact test

* Death occurred in one infant who enrolled but died before receiving any study oil

Table 3

DHA levels by study group compared to term peers

Group	Baseline	Full-Feedings	Discharge
Placebo supplemented preterm	2.91 (0.45) mol%	2.83 (0.50) mol%	2.87 (0.50) mol% *
DHA supplemented preterm	2.88 (0.68) mol%	3.03 (0.54) mol%	3.55 (0.44) mol% *†
Term	4.31 (0.95) mol%		

Results expressed as mean (SD) mol%; p<0.001.

* Groups were compared to term reference peers via ANOVA model

† Placebo vs. DHA supplemented comparison via linear mixed models