GROWTH AND DEVELOPMENT OF PRETERM INFANTS FED INFANT FORMULAS CONTAINING DOCOSAHEXAENOIC ACID AND ARACHIDONIC ACID

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Objectives To evaluate safety and benefits of feeding preterm infants formulas containing docosahexaenoic acid (DHA) and arachidonic acid (ARA) until 92 weeks postmenstrual age (PMA), with follow-up to 118 weeks PMA.

Study design This double-blinded study of 361 preterm infants randomized across three formula groups: (1) control, no supplementation; (2) algal-DHA (DHA from algal oil, ARA from fungal oil); and (3) fish-DHA (DHA from fish oil, ARA from fungal oil). Term infants breast-fed ≥4 months (n = 105) were a reference group. Outcomes included growth, tolerance, adverse events, and Bayley development scores.

Results Weight of the algal-DHA group was significantly greater than the control group from 66 to 118 weeks PMA and the fish-DHA group at 118 weeks PMA but did not differ from term infants at 118 weeks PMA. The algal-DHA group was significantly longer than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks PMA but did not differ from term infants from 79 to 118 weeks PMA. Supplemented groups had higher Bayley mental and psychomotor development scores at 118 weeks PMA than did the control group. Supplementation did not increase morbidity or adverse events.

Conclusions Feeding formulas with DHA and ARA from algal and fungal oils resulted in enhanced growth. Both supplemented formulas provided better developmental outcomes than unsupplemented formulas. *(J Pediatr 2005;146: 461-8)*

Exogenous sources of the long-chain omega-3 and omega-6 polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA), respectively, are important for premature infants. DHA and ARA accumulate rapidly in the developing brain during the last trimester of gestation,[1] and preterm infants are deprived of in utero accretion. Supplementing preterm formula with DHA and ARA at human milk levels results in circulating levels of these fatty acids similar to those seen in preterm infants fed breast milk.[2] DHA supplementation of preterm formula has been associated with accelerated visual maturation[3-5] and improved mental development.[6-8]

Some early studies of feeding preterm formula with fish oil providing DHA and another omega-3 fatty acid, eicosapentaenoic acid (EPA), but no ARA resulted in reduced growth,[5,9,10] which was associated with reduced ARA status of infants in one study.[9,11] EPA and its metabolites compete with and have other antagonistic effects on ARA and some of its metabolic effects.[12] Several subsequent studies of preterm formulas containing ARA as well as DHA found no consistent differences in growth compared with unsupplemented formulas,[13-16] suggesting that balanced addition of omega-6 and omega-3 long-chain fatty acids addressed the reduced growth seen in earlier studies. However, one study[17] reported reduced growth at 18 months after term associated with preterm formula containing DHA, EPA, and ARA from egg lipids. A second study[18] found significantly higher weights in the first few months of life with preterm formula with single-cell algal

ANOVA	Analysis of variance	MDI	Mental Development Index
ARA	Arachidonic acid	PDI	Psychomotor Development Index
DHA	Docosahexaenoic acid	PMA	Postmenstrual age
EPA	Eicosapentaenoic acid		

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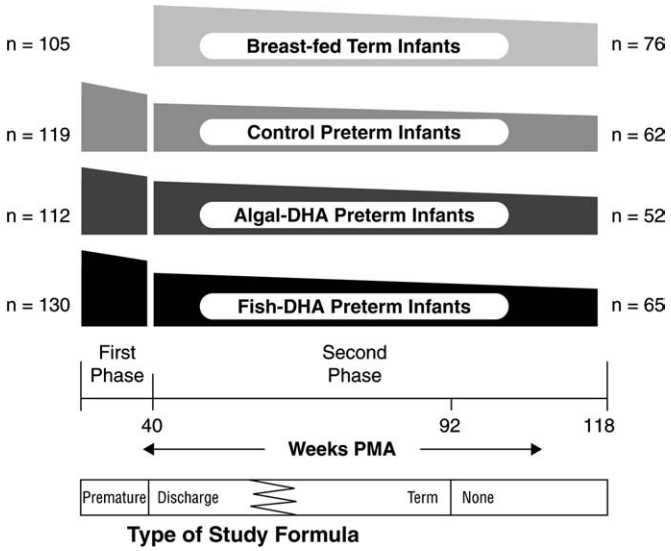

Figure 1. Study design with number enrolled to left and at completion to right. First phase was from start of enteral feeds to 40 weeks PMA; second phase was 40 to 118 weeks PMA. Protocol recommended feeding premature formula ≥14 days until hospital discharge, discharge formula until 53 weeks PMA, and term formula until 92 weeks PMA.

and fungal oils providing DHA and ARA but no EPA. Thus, questions remain about whether and how long-chain polyunsaturated fatty acids and specific sources of these fatty acids affect growth.[12]

Given the possible benefits of providing DHA and ARA to preterm infants and the growth concerns raised by some early trials, we considered it imperative to carefully evaluate clinical performance of formulas containing potential commercial sources of these fatty acids. Therefore, we conducted a large, double-blinded, randomized, controlled trial to assess the safety and efficacy of feeding preterm infants premature, discharge, and term infant formulas supplemented with DHA from algal oil or fish oil and ARA from fungal oil until 92 weeks postmenstrual age (PMA; 12 months after term).

METHODS

Subjects

Infants were eligible for enrollment in the first phase of this two-phase, multisite study if gestational age was ≤35 weeks PMA and they had received <10 total days of enteral feedings of >30 mL/kg per day (Figure 1). Infants initially fed human milk were not enrolled unless formula was started within 10 days after completing the first day of human milk feeding. Exclusion criteria included congenital abnormalities of the gastrointestinal tract, hepatitis, hepatic or biliary pathology, necrotizing enterocolitis confirmed before enrollment, or history of underlying disease or congenital malformation likely to interfere with evaluation. Given the minimal exclusion criteria, the study included many infants with concomitant medical conditions related to prematurity.

Preterm subjects eligible for inclusion in the second phase met the following criteria: successful completion of the first phase, ≥80% of enteral intake from study formula during hospitalization, and 100% of caloric intake from study formula at completion of the first phase (40 weeks PMA). A protocol amendment implemented shortly after enrollment began excluded preterm infants with birth weight >1500 g to limit inclusion to very and extremely low birth weight infants. Healthy, appropriate-for-gestational-age term infants (38 to 42 weeks gestational age) who were to be exclusively breast-fed for ≥4 months were enrolled as a reference group between birth and 4 weeks of age. Institutional review boards at each site reviewed and approved the protocol and procedures. Parents/guardians of all infants provided written informed consent.

Design

Infants enrolled in the first phase of this prospective, randomized, double-blinded, controlled study were stratified by birth weight (<1000 g, 1000 to 1500 g, and >1500 g initially and <1000 g and 1000 to 1500 g after the protocol amendment) and sex. Computer-generated random assignment schedules assigned infants to 1 of 3 study formula groups: (1) control, formulas with no added DHA or ARA; (2) algal-DHA, formulas with 17 mg DHA/100 kcal from algal oil and 34 mg ARA/100 kcal from fungal oil (Martek Biosciences, Columbia, MD); or (3) fish-DHA, formulas with 17 mg DHA/100 kcal from tuna fish oil (Roche Vitamins Inc, Parsippany, NJ) and 34 mg ARA/100 kcal from fungal oil. These levels of DHA and ARA (Table I) are similar to median worldwide amounts reported for mature human milk of approximately 0.3% by weight of fatty acids as DHA and 0.6% as ARA.[19,20] Subjects from the first phase who were eligible for enrollment in the second phase at 40 weeks PMA remained in their assigned formula group throughout the study.

Each study group was provided with premature (24 kcal/oz), discharge (22 kcal/oz), and term (20 kcal/oz) ready-to-use formulas, with the only differences being the polyunsaturated fatty acid profiles due to absence of DHA and ARA in control formulas and the sources of DHA in the supplemented formulas. The algal-DHA formulas were similar in ingredient and nutrient composition to Enfamil Premature LIPIL with Iron, EnfaCare LIPIL, and Enfamil LIPIL with Iron (Mead Johnson & Company, Evansville, IN). The protocol recommended feeding premature formula ≥14 days until at or near hospital discharge, discharge formula to 53 weeks PMA (3 months after term), and term formula to 92 weeks PMA (12 months after term). However, investigators were allowed discretion in selecting the formula type to meet the nutritional needs of each infant. Study formulas were to be the sole source of nutrition for preterm subjects until 57 weeks PMA (4 months after term) and the primary source of nutrition until 92 weeks PMA. Information was collected on which type of formula (premature, discharge, or term) each infant was consuming at 40, 44, 48, 53, and 57 weeks PMA. Study formula was stopped at 92 weeks PMA. Subjects in the second

Table I. Polyunsaturated fatty acid composition of study formulas (% by weight of total fatty acids)

Fatty acid	Study group	Preterm formula			Discharge formula			Term formula		
		Control	Algal-DHA	Fish-DHA	Control	Algal-DHA	Fish-DHA	Control	Algal-DHA	Fish-DHA
18:2n-6 Linoleic		18.7	18.6	18.6	19.5	19.4	19.4	17.5	17.2	17.1
18:3n-3 α-linolenic		2.4	2.4	2.4	2.4	2.4	2.4	1.68	1.65	1.65
20:4n-6 ARA		0.0	0.67	0.67	0.0	0.64	0.64	0.0	0.64	0.64
20:5n-3 EPA		0.0	0.0	0.10	0.0	0.0	0.10	0.0	0.0	0.10
22:6n-3 DHA		0.0	0.33	0.33	0.0	0.32	0.32	0.0	0.32	0.32

phase were monitored until 118 weeks PMA (18 months after term).

Growth, Intake, and Tolerance

Weight, length, and head circumference were measured by standardized procedures. All infants were assessed at birth and at 40, 44, 48, 53, 57, 66, 79, 92, and 118 weeks PMA. For preterm infants, growth data were collected weekly before hospital discharge and enteral intake and formula tolerance were recorded daily during hospitalization. Parents provided 24-hour diet and tolerance data at 40, 44, 48, 53, and 57 weeks PMA. Similar data were collected for the breast-fed term reference subjects at 44, 48, 53, and 57 weeks PMA.

Laboratory Measurements

Blood samples were collected from preterm subjects by heel prick or venipuncture at 57 weeks PMA, the age when exclusive formula feeding ended. Analyses included hematology; serum glucose, cholesterol, high-density lipoproteins, triglyceride, mineral, and electrolyte measurements; and liver and kidney function tests.

General Development

Trained testers at each site who were blinded to infants' study group assignments administered the Bayley Scales of Infant Development II[21] Mental Development Index (MDI) and Psychomotor Development Index (PDI) to all infants at 118 weeks PMA (18 months after term).

Morbidity and Adverse Events

Concomitant medical conditions (categorized by ICD-9-CM codes[22]) and use of concomitant therapies and medications were recorded for preterm infants during hospitalization. Detailed information was collected for specific conditions related to prematurity, including intraventricular hemorrhage, necrotizing enterocolitis (using modified Bell staging criteria), sepsis (confirmed by culture) or suspected sepsis, bronchopulmonary dysplasia (defined as requiring oxygen at 36 weeks PMA with severe or chronic changes to the lungs as seen on chest radiographs), and retinopathy of prematurity. All subjects were monitored for the occurrence of adverse events throughout the study. Investigators documented the occurrence and clinical outcome of each event.

Sample Size and Statistical Methods

The primary outcomes of this study were weights at 57 and 92 weeks PMA. A sample size of 58 infants per group would provide 80% power to detect a 500 g difference in weight among the study formula groups at 57 weeks PMA (SD = 950 g, $\alpha = 0.05$, 2-tailed); 31 infants per group were required to identify an 870 g difference at 92 weeks PMA (SD = 1200 g, $\alpha = 0.05$, 2-tailed). Anthropometric measurements at birth and subsequent study visits were analyzed by using analysis of variance (ANOVA). Study site, feeding regimen, and sex were included in the ANOVA models used to evaluate growth parameters. Bayley scores were analyzed by using an ANOVA model, including terms for study site and feeding regimen. The Van Elteren test, blocked for study site, was used to analyze laboratory measurements. Categoric variables were analyzed by using the Fisher exact test. Unadjusted pairwise comparisons were performed if initial tests were significant ($P < .05$).

RESULTS

Infant Characteristics

Preterm infants (n = 361) were enrolled in the first phase and randomly assigned to study formula groups (119 control, 112 algal-DHA, 130 fish-DHA). The fish-DHA group had significantly lower mean weight and head circumference at birth compared with the control and algal-DHA groups (Table II). The mean gestational ages at birth and at first consumption of study formula for the fish-DHA group also were less than those of the control group. The fish-DHA group had a somewhat ($P = .052$) higher incidence of multiple (>2) births (27% vs 15% for control and 16% for algal-DHA) and fewer twins (15% vs 22% for control and 27% for algal-DHA). There were no significant differences among groups in distribution by sex, birth weight categories, or racial groups (data not shown). Fifty-six infants (21 control, 17 algal-DHA, 18 fish-DHA) in the first phase discontinued before 40-week PMA. The most common reasons for discontinuation were formula intolerance (n = 15), medical complications unrelated to the study (n = 13), and parental request (n = 11). There were no differences among groups in discontinuation rates or distribution of reasons for discontinuation.

Table II. Characteristics of study participants

Infant characteristics	Study group		
	Control (n = 119)	Algal-DHA (n = 112)	Fish-DHA (n = 130)
Infants enrolled in first phase			
Birth weight (g)†	1215 ± 33	1189 ± 34	1107 ± 31‡
(range)	(455–2340)	(490–2363)	(430–2280)
No. <1000g/1000–1500 g/>1500 g birth weight	30/73/16	27/77/8	44/79/7
Birth length (cm)†	37.5 ± 0.4	37.4 ± 0.4	36.6 ± 0.4
(range)	(27–47)	(28–48)	(28–46)
Birth head circumference (cm)†	26.7 ± 0.3	26.6 ± 0.3	25.9 ± 0.2‡
(range)	(19–41)	(21–38)	(21–33)
Gestational age at birth (weeks PMA)†	29.6 ± 0.3	29.4 ± 0.3	28.8 ± 0.2§
(range)	(23–35)	(23–35)	(24–34)
Age study formula first consumed (weeks PMA)†	31.2 ± 0.2	30.9 ± 0.2	30.5 ± 0.2§
(range)	(24–36)	(25–36)	(25–34)
No. male/female	67/52	54/58	73/57

	Control (n = 83)	Algal-DHA (n = 72)	Fish-DHA (n = 90)	Breast-fed term (n = 105)
Infants enrolled in second phase				
Birth weight (g)†	1179 ± 35	1207 ± 37	1110 ± 33	3483 ± 39
(range)	(455–2340)	(490–1590)	(430–1499)	(2724–4485)
No. <1000g/1000–1500 g/>1500 g birth weight	21/60/2	16/55/1	31/59/0	0/0/105
Birth length (cm)†	37.5 ± 0.5	37.4 ± 0.5	36.7 ± 0.4	50.8 ± 0.2
(range)	(27–46)	(28–43)	(28–44)	(46–57)
Birth head circumference (cm)†	26.6 ± 0.3	26.9 ± 0.4	26.0 ± 0.3	34.5 ± 0.1
(range)	(19–41)	(21–38)	(21–30)	(32–39)
Gestational age at birth (preterms; weeks PMA)†	29.7 ± 0.3	29.7 ± 0.3	28.9 ± 0.3	—
(range)	(24–35)	(23–34)	(24–33)	
Age study formula first consumed (weeks PMA)†	31.4 ± 0.3	31.2 ± 0.3	30.7 ± 0.2	—
(range)	(27–36)	(26–36)	(25–34)	
No. male/female	45/38	34/38	49/41	48/57

†Mean ± SEM (range).
‡$P < .05$, Fish-DHA vs control, algal-DHA.
§$P < .05$, Fish-DHA vs control.

Sixty preterm infants (15 control, 23 algal-DHA, 22 fish-DHA) completing the first phase were not enrolled in the second phase for the following reasons: <80% of enteral feedings during hospitalization or <100% at 40 weeks PMA from study formula (n = 27); birth weight >1500 g (n = 19); formula intolerance (n = 6); parent (n = 4) or physician (n = 3) elected withdrawal; and >7 consecutive days off study formula (n = 1).

Two hundred forty-five preterm infants (83 control, 72 algal-DHA, 90 fish-DHA) and 105 breast-fed term infants were enrolled in the second phase. Three preterm infants with birth weight >1500 g who started the second phase before the protocol amendment remained in the study. Among the preterm groups, there were no significant differences in weight, length, head circumference, or gestational age at birth; gestational age when study formula was first consumed; sex or birth weight category (Table II); or racial distribution (data not shown). Compared with the algal-DHA group, the fish-DHA group had a higher incidence of multiple (>2) births (30% for fish-DHA vs 17% for algal-DHA) and a lower incidence of twins (11% for fish-DHA and 29% for algal-DHA; $P < .01$), with the control group intermediate (18% multiples and 20% twins). The distribution of infants across types of formula (premature, discharge, or term) did not differ among preterm groups at any time (data not shown). A total of 179 preterm (62 control, 52 algal-DHA, 65 fish-DHA) and 76 term infants completed the second phase. Discontinuation rates did not differ among study groups. Among the preterm groups, there were no differences in reasons for discontinuing the study during the second phase.

Growth

Mean weight, length, and head circumference growth rates in the first phase did not differ among preterm groups

(data not shown). Mean weight, length, and head circumference also did not differ among the three preterm groups at the completion of the first phase (40 weeks PMA; data not shown) or at the start of the second phase (Figure 2; data not shown for head circumference). Mean weights at the beginning of the second phase were (mean ± SEM) 2964 ± 56 g for the control group, 2954 ± 58 g for the algal-DHA group, and 3063 ± 51 g for fish-DHA group; mean lengths were 47.7 ± 0.31 cm for the control group, 47.6 ± 0.33 cm for the algal-DHA group, and 47.5 ± 0.28 cm for the fish-DHA group. Breast-fed term infants had greater mean weights (Figure 2, upper panel) than all preterm groups at all ages evaluated except the algal-DHA group at 118 weeks PMA. The algal-DHA group had greater mean weights than the control group at 66, 79, 92, and 118 weeks PMA and the fish-DHA group at 118 weeks PMA. Mean body length was greater for breast-fed term infants (Figure 2, lower panel) than all preterm groups at 40, 44, 48, 53, 57, and 66 weeks PMA and greater than the control and fish-DHA groups but not the algal-DHA group, at 79, 92, and 118 weeks PMA. The algal-DHA group had greater mean lengths than the control group at 48, 79, and 92 weeks PMA and the fish-DHA group at 57, 79, and 92 weeks PMA. There were no differences in mean head circumference (data not shown) among the preterm and breast-fed term groups through 66 weeks PMA. Small differences ($P < .05$) were noted at 79 weeks PMA, with the algal-DHA group greater than the fish-DHA group and breast-fed term infants greater than the control and fish-DHA groups, and at 92 weeks PMA, with breast-fed term infants greater than the control and fish-DHA groups. Mean head circumference of the breast-fed term infants (mean ± SEM; 48.2 ± 0.29 cm) was greater than that of the control group (47.5 ± 0.30 cm; $P = .052$) and fish-DHA group (47.2 ± 0.32 cm; $P = .004$) at 118 weeks PMA, whereas the algal-DHA group (47.7 ± 0.36 cm) did not differ from any other group.

Intake and Tolerance

There were no differences in caloric intake from formula, daily gastric residuals, stool frequency, stool consistency, or abdominal distention among the preterm groups during hospitalization (data not shown). Based on parental reports, the algal-DHA group consumed more formula than did the fish-DHA group at 40 weeks PMA (mean ± SEM; 199.8 ± 8.5 vs 175.4 ± 7.5 mL/kg per day; $P < .01$) and the control and fish-DHA groups at 48 weeks PMA (215.9 ± 7.7 vs 188.3 ± 7.4 and 189.8 ± 6.9 mL/kg per day, respectively; $P < .01$), but there were no differences among preterm groups in reported mean formula intakes at 44, 53, or 57 weeks PMA. There were no differences among preterm groups with respect to parental reports of fussiness, diarrhea, or constipation at any time during the study (data not shown). Parents reported a greater incidence ($P < .05$) of "more gas than usual" for the algal-DHA group than the control group at 40 and 44 weeks PMA and for the fish-DHA group than the control group at 48 weeks PMA, but there were no differences at 53 or 57 weeks PMA.

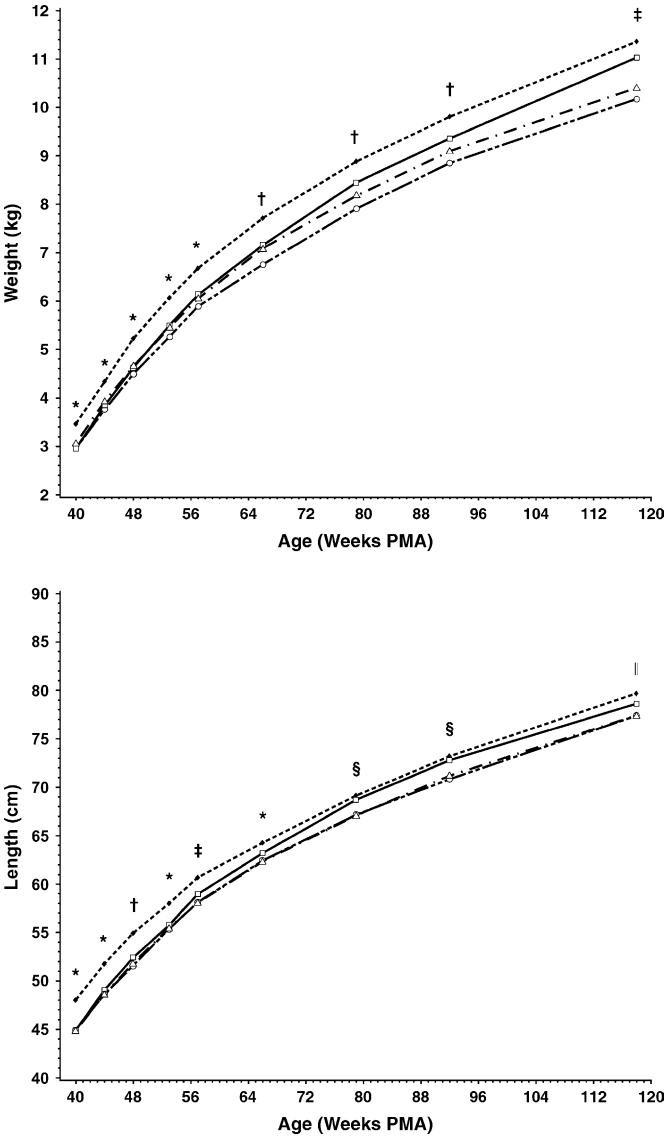

Figure 2. Mean weight (*upper panel*) and length (*lower panel*) in the second phase, from 40 to 118 weeks PMA. ♦, Breast-fed term group; ○, control group; □, algal-DHA group; Δ, fish-DHA group. *Upper panel* (weight): *Breast-fed term > control, algal-DHA, fish-DHA; †breast-fed term > control, algal-DHA, fish-DHA, and algal-DHA > control; ‡breast-fed term, algal-DHA > control, fish-DHA. *Lower panel* (length): *Breast-fed term > control, algal-DHA, fish-DHA; †breast-fed term > control, algal-DHA, fish-DHA, and algal-DHA > control; ‡breast-fed term > control, algal-DHA, fish-DHA, and algal-DHA > fish-DHA; §breast-fed term, algal-DHA > control, fish-DHA; ‖breast-fed term > control, fish-DHA (all at $P < .05$).

General Development

Mean Bayley MDI and PDI scores of the breast-fed term infants at 118 weeks PMA (18 months after term) were near the reference norm of 100 and significantly higher than those for any preterm group (Figure 3). The algal-DHA and fish-DHA groups had higher MDI and PDI scores than did the control group. Similar differences were seen in a subanalysis of the Bayley scores in which infants with organic

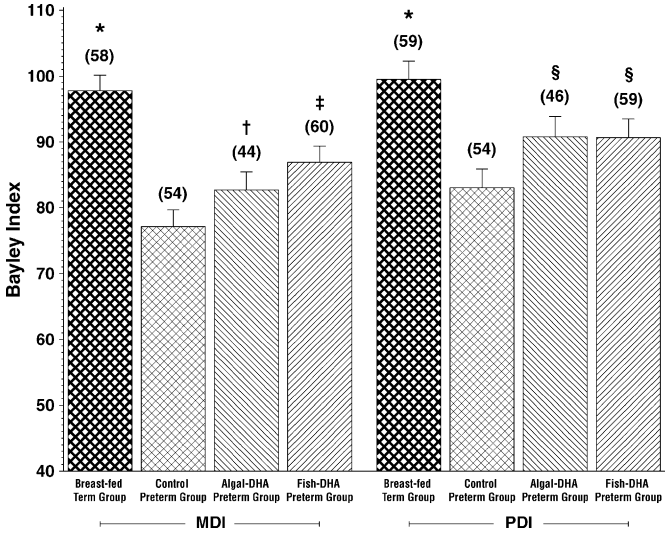

Figure 3. Mental Development Index (MDI) and Psychomotor Development Index (PDI) of the Bayley Scales of Infant Development II at 118 weeks PMA [mean ± SEM (n)]. *Breast-fed term > control, algal-DHA, fish-DHA ($P < .05$); †algal-DHA > control ($P = .056$); ‡fish-DHA > control ($P < .05$); §algal-DHA, fish-DHA > control ($P < .05$).

brain disease (eg, hydrocephalus, periventricular leukomalacia) were excluded (data not shown).

Safety Indexes

The only significant differences across 180 ICD-9-CM diagnostic categories[22] diagnosed during initial hospitalization of preterm infants were "other conditions of the brain" (control, 9% vs fish-DHA 0%, $P < .001$) and "nonspecific low blood pressure readings" (algal-DHA, 4% vs fish-DHA, 0%; $P = .019$). Occurrences of specific medical conditions related to prematurity (Table III) were similar among groups during the first phase, with one exception. Incidence of intraventricular hemorrhage was similar at study enrollment (data not shown), but the algal-DHA group had a significantly lower occurrence at the end of the first phase. Among preterm groups, there were no differences in adverse events in the first phase and no differences in adverse events for any body system except the nervous system (control, 16% vs fish-DHA, 6%; $P = .04$) during the study. Two infants in the control group and 3 in the fish-DHA group died during initial hospitalization. Two infants in the control group died during the second phase of the study. Study site clinical investigators determined that the deaths were not related to study formula. There were significant differences among 3 of the 31 laboratory measurements: higher mean corpuscular hemoglobin for the fish-DHA group than for the control group (27.6 vs 27.0 pg/cell; $P = .03$); higher total cholesterol for the fish-DHA group than for the control and algal-DHA groups (3.85, 3.43, 3.48 mmol/L, respectively; $P < .05$); and lower serum potassium for the fish-DHA group than the control group (5.0 vs 5.3 mmol/L; $P = .003$).

DISCUSSION

This clinical trial demonstrated that feeding infant formulas with median worldwide human milk levels of DHA and ARA[19,20] from single-cell algal and fungal oils can enhance growth of premature infants. A previous study[18] found that very low birth weight (range, 846 to 1560 g; mean, ~1250 g) infants fed preterm formula with similar levels of DHA and ARA from the same sources gained weight faster during initial hospitalization than infants fed unsupplemented formula (34.7 vs 30.7 g/d). In addition, preterm infants fed the formula with DHA and ARA in the previous study had weights not different from breast-fed term infants at 48 and 57 weeks PMA, whereas those fed either unsupplemented formula or formula with DHA but no ARA remained significantly smaller than the term reference group at these ages. Our study did not find an effect of DHA and ARA on weight gain during initial hospitalization, which may be related to the inclusion of more extremely low birth weight infants with greater concomitant medical complications. It did demonstrate, however, longer-term growth enhancement associated with the tested levels of DHA and ARA from the algal and fungal oil sources. The very and extremely low birth weight infants fed formulas with DHA and ARA from algal and fungal oils from the start of enteral feeding to 12 months after term achieved body weights and lengths comparable to breast-fed term infants by 18 months after term, whereas infants fed unsupplemented formulas or formulas with DHA from fish oil did not. These results are of considerable importance because very and extremely low birth weight preterm infants remain at risk for subnormal weight and height through childhood and perhaps into adulthood.[23-25]

The mechanism for the increase in growth with DHA and ARA from algal and fungal oils is not known. Innis et al[18] reported significant positive correlations between red blood cell ARA levels and weight and length of preterm infants at 40, 48, and 57 weeks PMA and speculated on potential mechanisms. Lapillonne et al[12] also discussed mechanisms whereby DHA, EPA, and/or ARA may influence growth, including effects on eicosanoid production, gene expression, and membrane characteristics. In the current study, increased weight and length were seen with DHA from algal oil but not from fish oil, which provides some EPA in addition to DHA. Whether EPA played a role in the lower growth seen with the fish-DHA formulas compared with the algal-DHA formulas, however, is not clear. In the first phase of the study, the fish-DHA group had a lower mean birth weight than the control or algal-DHA groups, possibly related to more multiple (>2) births, but the mean birth weights of preterm infants continuing into the second phase did not differ among groups. Parents reported that infants in the algal-DHA group consumed more formula than those in the fish-DHA group at 40 weeks PMA and those in the control and fish-DHA groups at 48 weeks PMA, but intakes did not differ significantly among preterm groups at other ages.

Other studies of preterm infants fed formulas with DHA and ARA have not found enhanced growth, which may

Table III. Occurrence of specific medical conditions in first phase (initial hospitalization)

	Study group					
	Control		Algal-DHA		Fish-DHA	
Medical condition	n	(%)	n	(%)	n	(%)
Intraventricular hemorrhage	32	(29)	14*	(13)	33	(27)
Necrotizing enterocolitis†	3	(3)	6	(5)	7	(5)
Confirmed sepsis	16	(13)	19	(17)	19	(15)
Bronchopulmonary dysplasia‡	17	(15)	16	(15)	21	(17)
Retinopathy of prematurity	31	(42)	35	(47)	53	(58)

*$P < .01$, Algal-DHA vs control, fish-DHA.
†Stage II or III.
‡Defined as the requirement for oxygen at 36 weeks PMA with severe or chronic changes to the lung demonstrated on chest radiography.

be related to the levels and/or sources of the fatty acids or other aspects of study population characteristics or study design. Foreman-van Drongelen et al[13] and Vanderhoof et al[14,15] fed formulas with algal and fungal-oil sources of DHA and ARA, at similar levels (0.3% to 0.35% by weight of total fatty acids as DHA and 0.5% to 0.61% as ARA), to 3 and 2 months after term, respectively. Both studies, however, included preterm infants with higher average birth weights of ~1500 g. O'Connor et al[16] included smaller (mean birth weight, ~1300 g; range, 750 to 1805 g) and less healthy infants and fed test formulas to 12 months after term. The formulas studied, however, used lower levels of DHA (0.15% to 0.27%) and ARA (0.41% to 0.43%); DHA sources were fish oil and egg-derived triglycerides, with ARA from fungal oil. Fewtrell et al[17] studied preterm formula with egg lipid providing 0.17% DHA, 0.04% EPA, and 0.31% ARA. Study formulas were fed to preterm infants with mean birth weights of ~1340 g for only 31 to 33 days on average. Although the first three studies above[13-16] did not find consistent effects of DHA and ARA on preterm infant growth, Fewtrell et al[17] reported significant reductions in weight and length at 18 months after term associated with supplementation. Whether this negative effect on growth was related to the source of DHA and ARA or to some other aspect of the study formula, population, or design is unknown. However, the studies with the algal and fungal oils noted above,[13-16,18] as well as the current study, show no consistent negative effects on preterm infant growth and suggest the potential for growth enhancement of smaller premature infants.

In addition to the growth decrements associated with prematurity, very and extremely low birth weight infants have an increased risk of neurologic deficits and overall greater morbidity.[23-25] Thus, it was not unexpected that the breast-fed term group would have significantly higher Bayley scores than all groups of preterm infants. The more important finding is that the addition of these levels of DHA and ARA to the diets of preterm infants improved their mental and psychomotor development at 18 months after term, compared with preterm infants fed unsupplemented formula. Other investigators have not found significant differences in MDI or PDI scores at 12[16] or 18[17] months after term with supplemented formulas, although results suggested that smaller[16] or more preterm[17] infants might benefit from supplementation. Studies of term infants also show that the relation between DHA and ARA supplementation and developmental benefits may depend on levels, sources, or other differences in experimental design.[26-28]

Despite hypothetical concerns about adding DHA and ARA to formulas for preterm infants[23,29] such as potential interference with host defense mechanisms or impact on hemostasis, we found no increase in morbidity associated with supplementation. Our analysis of a wide spectrum of clinical data, including serum chemistry and hematology values and incidence and severity of medical conditions related to prematurity, found no safety issues related to the supplemented formulas. The finding of a significantly lower incidence of intraventricular hemorrhage in the algal-DHA group was unexpected. It is of interest that this group also had a higher incidence of nonspecific low blood pressure readings. Other investigators[13-18] also did not report increases in incidence of adverse events among preterm infants fed formulas with DHA and ARA.

Our study is unique in comparing algal oil and fish oil sources of DHA, each in combination with ARA from fungal oil for the long-term feeding of preterm infants, including those with extremely low birth weights and concurrent medical conditions associated with prematurity. Both combinations of long-chain polyunsaturated fatty acid sources supported significant developmental benefits for these at-risk infants. We also confirmed the growth-promoting effect of algal DHA plus fungal ARA, as first reported in the study by Innis et al.[18] Overall, these results demonstrate that feeding formulas supplemented with DHA and ARA from algal and fungal oils at these levels to 12 months after term results in meaningful growth and development benefits for formula-fed preterm infants, even beyond the first year of life.

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REFERENCES

1. Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Hum Dev 1980;4:121-9.

2. Clandinin MT, Van Aerde JE, Parrott A, Field CJ, Euler AR, Lien EL. Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in preterm infant formulas: fatty acid composition of erythrocyte membrane lipids. Pediatr Res 1997;42:819-25.

3. Birch EE, Birch DG, Hoffman DR, Uauy R. Dietary essential fatty acid supply and visual acuity development. Invest Ophthalmol Vis Sci 1992;33: 3242-53.

4. Carlson SE, Werkman SH, Rhodes PG, Tolley EA. Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. Am J Clin Nutr 1993;58:35-42.

5. Carlson SE, Werkman SH, Tolley EA. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. Am J Clin Nutr 1996;63:687-97.

6. Carlson SE, Werkman SH, Peeples JM, Wilson WM III. Growth and development of premature infants in relation to ω3 and ω6 fatty acid status. World Rev Nutr Diet 1994;75:63-9.

7. Carlson SE, Werkman SH. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. Lipids 1996;31: 85-90.

8. Werkman SH, Carlson SE. A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months. Lipids 1996;31: 91-7.

9. 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;27:901-7.

10. Ryan AS, Montalto MB, Groh-Wargo S, Mimouni F, Sentipal-Walerius J, Doyle J, et al. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. Am J Human Biol 1999;11: 457-67.

11. Carlson SE, Werkman SH, Peeples JM, Cooke RJ, Tolley EA. Arachidonic acid status correlates with first year growth in preterm infants. Proc Natl Acad Sci U S A 1993;90:1073-7.

12. Lapillonne A, Clarke SD, Heird WC. Plausible mechanisms for effects of long-chain polyunsaturated fatty acids on growth. J Pediatr 2003;143: S9-16.

13. Foreman-van Drongelen MMHP, van Houwelingen AC, Kester ADM, Blanco CE, Hasaart THM, Hornstra G. Influence of feeding artificial-formula milks containing docosahexaenoic and arachidonic acids on the postnatal long-chain polyunsaturated fatty acid status of healthy preterm infants. Br J Nutr 1996;76:649-67.

14. Vanderhoof J, Gross S, Hegyi T, Clandinin T, Porcelli P, DeCristofaro J, 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;29:318-26.

15. Vanderhoof J, Gross S, Hegyi T. A multicenter long-term safety and efficacy trial of preterm formula supplemented with long-chain polyunsaturated fatty acids. J Pediatr Gastroenterol Nutr 2000;31:121-7.

16. O'Connor DL, Hall R, Adamkin D, Auestad N, Castillo M, Connor WE, et al. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. Pediatrics 2001;108:359-71.

17. Fewtrell MS, Morley R, Abbott RA, Singhal A, Isaacs EB, Stephenson T, et al. Double-blind, randomized trial of long-chain polyunsaturated fatty acid supplementation in formula fed to preterm infants. Pediatrics 2002;110: 73-82.

18. Innis SM, Adamkin DH, Hall RT, Kalhan SC, Lair C, Lim M, et al. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula. J Pediatr 2002;140:547-54.

19. Innis SM. Human milk and formula fatty acids. J Pediatr 1992;120: S56-61.

20. Koletzko B, Thiel I, Abiodun PO. The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;120:S62-70.

21. Bayley N. Bayley Scales of Infant Development. 2nd edition. San Antonio, Texas: The Psychological Corporation, Harcourt Brace & Company; 1993.

22. US Department of Health and Human Services. In: Daly S, Adam R, eds. Generic ICD-9-CM. Hospital Version. Reno, Nevada: Channel Publishing, Ltd; 1999.

23. Hay WW Jr, Lucas A, Heird WC, Ziegler E, Levin E, Grave GD, et al. Workshop summary: nutrition of the extremely low birth weight infant. Pediatrics 1999;104:1360-8.

24. Saigal S, Stoskopf BL, Streiner DL, Burrows E. Physical growth and current health status of infants who were of extremely low birth weight and controls at adolescence. Pediatrics 2001;108:407-15.

25. Hack M, Flannery DJ, Schluchter M, Cartar L, Borawski E, Klein N. Outcomes in young adulthood for very-low-birth-weight infants. N Engl J Med 2002;346:149-57.

26. Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG. A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Dev Med Child Neurol 2000; 42:174-81.

27. Auestad N, Halter R, Hall RT, Blatter M, Bogle ML, Burks W, et al. Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. Pediatrics 2001;108:372-81.

28. Auestad N, Scott DT, Janowsky JS, Jacobsen C, Carroll RE, Montalto MB, et al. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. Pediatrics 2003;112:e177-83.

29. Heird WC. Biological effects and safety issues related to long-chain polyunsaturated fatty acids in infants. Lipids 1999;34:207-14.

APPENDIX

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