

Safety and efficacy of omega-3 fatty acids in the nutrition of very low birth weight infants: Soy oil and marine oil supplementation of formula

Ricardo Uauy, MD, PhD, Dennis R. Hoffman, PhD, Eileen E. Birch, PhD, David G. Birch, PhD, David M. Jameson, PhD, and Jon Tyson, MD

From the Clinical Nutrition Unit, Institute of Nutrition and Food Technology, University of Chile, Santiago; the Division of Neonatology, Department of Pediatrics, and the Department of Ophthalmology, University of Texas Southwestern Medical Center at Dallas; the Retina Foundation of the Southwest, Dallas, Texas; and the Departments of Biochemistry and Biophysics, University of Hawaii at Manoa, Honolulu

Because formula-fed preterm infants may be at risk of ω 3 essential fatty acid deficiency, we tested experimental formulas supplemented with soy oil to provide α -linolenic acid or marine oil to provide preformed ω 3 long-chain polyunsaturated fatty acids at a level comparable to that of human milk. This report addresses the effect of feeding formula supplemented with soy oil or with soy and marine oils on growth, clinical tolerance, coagulation test results, changes in erythrocyte membrane fluidity, and plasma concentrations of vitamins A and E in very low birth weight infants from 30 to 57 weeks of postconceptional age. "Healthy" preterm infants were maternally selected to receive human milk or selected at random to receive commercial ready-to-feed liquid formula, which provided limited ω 3 fatty acid, or experimental formulas supplemented with soy oil or soy and marine oils. Results of this study indicate that formula enriched with soy oil or soy and marine oils containing preformed ω 3 long-chain polyunsaturated fatty acids does not induce abnormalities in growth, clotting function, erythrocyte membrane fluidity, or vitamin A or E levels in healthy very low birth weight preterm infants. Additional studies to evaluate safety in a representative preterm population are required. (J PEDIATR 1994;124:612-20)

Lipids have been considered a key postnatal energy source for growth, metabolism, and muscle activity. The structural role of long-chain fats and the functional correlates of specific fatty acids are being increasingly recognized.^{1,2} The importance of essential fatty acids as dietary precursors for

eicosanoid and docosanoid formation has also been reported.^{3,4}

Animals lack the enzymes necessary to form ω 3 or ω 6

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Reprint request: Ricardo Uauy, MD, PhD, Retina Foundation of the Southwest, 9900 North Central Expressway, Suite 400, Dallas, TX 75231.

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ANOVA	Analysis of variance
EFA	Essential fatty acid
LCPUFA	Long-chain polyunsaturated fatty acid
(V)LBW	(Very)low birth weight
20:4 ω 6	Arachidonic acid
22:6 ω 3	Docosahexaenoic acid
20:5 ω 3	Eicosapentaenoic acid
18:2 ω 6	Linoleic acid
18:3 ω 3	α -Linolenic acid

fatty acids and hence require the parent EFA in their diets.⁵ Microsomes in liver, brain, and other animal tissues are ca-

pable of further elongating and desaturating the parent EFAs, generating a family of compounds for each series. The competitive desaturation of the $\omega 3$, $\omega 6$, and $\omega 9$ series by Δ^6 desaturase is of major significance for EFA nutrition in the newborn infant because this is the rate-limiting step of the elongation-desaturation pathway.^{1, 6-8} Details of EFA metabolism and requirements can be found in published reviews.^{1, 5, 8-10}

Nearly 30 years ago Hansen et al.¹⁰ published a study of 428 infants fed milk formulations with varying amounts and kinds of fats and established linoleic acid as essential for normal infant nutrition. More recently, interest has focused on the potential requirements and benefits of the dietary $\omega 3$ EFA, α -linolenic acid.¹¹ Synthesis of eicosapentaenoic acid from α -linolenic acid in animals provides a precursor for series 3 eicosanoids, which commonly antagonize the arachidonate-derived series 2 eicosanoids responsible for the inflammatory response. Long-chain polyunsaturated fatty acids, such as docosahexaenoic acid, provide a specific structural environment within the phospholipid bilayer; thus membrane fatty acid composition may influence important functions such as ion or solute transport, receptor activity, and enzyme action.^{9, 12, 13}

Recent studies have attempted to characterize the biochemical and functional effects of dietary $\omega 3$ fatty acid deficiency. Animal models have been developed with purified 18:2 $\omega 6$ as the only dietary fat or with safflower or sunflower oil in which the amount of 18:3 $\omega 3$ was very low and the ratio of 18:2 $\omega 6$ to 18:3 $\omega 3$ was very high (approximately 250:1).¹⁴⁻¹⁶ Similarly low 18:3 $\omega 3$ content was found in powdered infant formulas currently in use in many parts of the world.¹⁷

Electrophysiologic studies in animals showed that the a wave of the electroretinogram, an index of photoreceptor activity, was significantly altered by dietary 18:3 $\omega 3$ deficiency.^{15, 16} The visual acuity of the $\omega 3$ fatty acid-deficient 8-week-old primates, measured by preferential looking response, was reduced in comparison with that in a control group.^{14, 15} A direct correlation between brain and erythrocyte membrane phospholipid 22:6 $\omega 3$ composition during deficiency was also established in animal studies.^{14, 18, 19} In contrast, rats fed diets containing the parent EFA, 18:3 $\omega 3$, had higher brain lipid levels of 22:6 $\omega 3$.^{16, 20}

During the past years, we and others have conducted studies to evaluate the effect of $\omega 3$ fatty acid supplementation in very low birth weight infants, examining the effects on plasma and tissue lipid composition,²⁰⁻²⁶ retinal electrophysiologic function,^{22, 26} and maturation of visual cortical function.^{22, 27} Preterm infants may be particularly vulnerable to $\omega 3$ fatty acid deficiency, given the increased need for fat accretion, the absence of fat storage, and the immaturity of fatty acid metabolism.^{6, 28-30} Preterm infants fed corn

oil- or soy oil-based formulas had lower $\omega 3$ LCPUFA in plasma and erythrocyte lipids than infants who received the soy oil-marine oil formula or human milk.^{22, 25} Electroretinographic, pattern-evoked potential, and psychophysical measures of visual function were comparable between the soy oil-marine oil group and the human milk group, but infants receiving either corn oil- or soy oil-based formula had altered visual development.^{22, 26, 27} A recent psychophysical study of visual acuity by Carlson et al.³¹ demonstrated a transient benefit of marine oil supplementation during early development.

Although the efficacy of marine oil supplementation on visual function has been addressed, questions persist concerning the safety of this nutritional intervention. The purpose of this communication is to report our findings on the safety of $\omega 3$ fatty acid supplementation with soy and marine oils in preterm infant formulas.

METHODS

Subjects. Infants with birth weights of 1000 to 1500 gm were eligible to enter the study if they tolerated enteral feedings (70 to 120 kcal/kg) and had no major neonatal morbidity by the tenth day of life. The final cohort analysis included only infants for whom the biochemical and anthropometric data collection had been completed. Infants fed their own mothers' milk from birth provided "reference standard" data for a preterm group fed human milk. Formula-fed infants were randomly assigned to one of three groups receiving varying amounts of $\omega 3$ fatty acids. These infants were matched with the group fed human milk by birth weight category (1000 to 1249 and 1250 to 1500 gm). They received routine clinical care as indicated by the staff of the Parkland Memorial Hospital Neonatal Intensive Care Unit, University of Texas Southwestern Medical Center, Dallas. Ethnic makeup was black (60%), white (24%), and Hispanic (16%). This study included an initial group of infants followed from entry to 40 weeks of postconception age. A follow-up phase began at 40 weeks and continued until 57 weeks. Although 83 infants were entered into the study by the tenth day of life, complete visual, anthropometric, and biochemical data had been collected for 70 infants at 40 weeks. By 57 weeks, complete data for a total of 52 infants had been obtained.

Clinical care was provided by attending and resident physicians who were familiar with the study but unaware of diet group assignments. The following problems likely to influence feeding or visual responses were used as exclusion criteria: respirator treatment for more than 7 days, clinically evident congenital infection or major malformations, gastrointestinal surgery with major resections, or grade III or IV intracranial hemorrhage (Papile classification). An

ophthalmologist assessed all infants for retinopathy at 35 to 36 weeks of postconceptional age. One infant with stage 3 retinopathy³² was excluded from the study. No infant had used a ventilator after day 5 or for more than 3 days. No infant had bronchopulmonary dysplasia as defined by oxygen use at 28 days and a chest radiograph compatible with chronic lung disease. Only five infants required blood transfusions after random assignment, and all transfusions were given at least 2 weeks before blood sampling; two infants received formula A (with corn oil), one received formula B (with soy oil), and two received formula C (with soy and marine oils). Post hoc analysis did not show evidence of an effect of blood transfusion on lipid compositional changes.

Written informed consent was obtained from parents of all infants. All infants received vitamin, mineral, and taurine supplements as part of our neonatal intensive care unit routine to meet present recommendations for VLBW infants. Once the feedings were well tolerated, infants were given 25 IU vitamin E per day for 14 days.

Diets. The preterm human milk group received their mothers' milk, which had been refrigerated or prefrozen and gently thawed in accordance with the University of Texas Southwestern Medical Center human milk banking protocol. The milk was supplemented with a standardized fat-free milk fortifier to ensure comparable macronutrient and micronutrient intake. For estimation of EFA intake, pooled composite samples of milk were obtained before feeding, stored at -30°C , and analyzed for total fatty acid composition. Considerable effort to promote breast-feeding was needed to support the human milk-fed group. Ten mothers were successful in establishing an adequate milk supply throughout the first 5 weeks of the study. One infant fed human milk was lost during follow-up because of parental noncompliance. During the first 5 weeks of the study, six infants received more than 75% of their energy intake from human milk, and four received between 50% and 75%. Consumption of human milk was $73\% \pm 7\%$ (mean \pm SEM) through the first 5 weeks of the study, $58\% \pm 9\%$ through 2 months, and $48\% \pm 9\%$ through 4 months. During periods of limited milk supply, formula C, was provided because its fatty acid profile most closely resembled that of human milk.

Infants whose mothers chose not to provide milk were randomly assigned to receive a formula. Formula groups A, B, and C were fed LBW formula containing protein, 2.4 gm/dl (whey/casein ratio, 60:40); carbohydrate, 8.9 gm/dl (lactose/glucose ratio, 40:60); and fat, 4.1 gm/dl, with varying amounts of EFA. Formula A, based on medium-chain triglycerides, coconut oil, and corn oil, provided primarily 18:2 ω 6 (24.2%) and 18:3 ω 3 (0.5%) as EFA and cor-

responded with commercial powdered premature formulas existing before 1987. Formula B, based on medium-chain triglycerides, coconut oil, and soy oil, supplied 18:2 ω 6 (20.8%) and 18:3 ω 3 (2.7%) and corresponded with liquid ready-to-feed premature formula. Formula C, an experimental product prepared especially for the study, was similar to formula B (18:2 ω 6, 20.8%; 18:3 ω 3, 2.7%; 20:4 ω 6, 0.1%; 20:5 ω 3, 0.65%; 22:6 ω 3, 0.35%) but was supplemented with marine oil (winterized, deodorized, stabilized menhaden oil provided by Zapata-Haynie Co., Reedville, Va.) to provide ω 3 LCPUFA, specifically 22:6 ω 3 at a level equivalent to that found in human milk (approximately 0.3%). In addition, some corn oil was included in formula C to obtain a level of 18:3 ω 3 comparable to that found in human milk. The term formulas used after infants' discharge from the nursery were adjusted to the needs of neonates born at ≥ 36 weeks of gestation (lower protein, caloric density, electrolytes, calcium, phosphorus, and trace minerals but higher iron content than the preterm formulation). The formulas were provided by Mead Johnson Nutritional Division (Evansville, Ind.). Details of the nutrient composition of the formulas are available on request; fatty acid compositions have been published.²⁵

Studies relative to oxidation of the lipids in these formulas were conducted in the food science laboratories of the formula manufacturer, with standard methods used to test peroxidation under accelerated conditions and under usual storage conditions. The results were well within accepted standards. The vitamin and mineral content of the formulas met recommendations of the American Association of Pediatrics for LBW neonates. Vitamin E content was adjusted to ensure at least 2 mg of α -tocopherol per gram of unsaturated fatty acid in the formulas.

During the follow-up to the adjusted postnatal age of 4 months, infants received formula or human milk as the sole source of fat. Cereals, fruit juices, or fruits were permitted according to routine practices and infant tolerance. These foods contain virtually no fat; therefore their inclusion should not affect the results of the study.

Clinical monitoring. Before discharge from the hospital, all VLBW neonates were monitored daily by the research nurse as supervised by one of us (R.U. or J.T.). Volume of intake, feeding tolerance, and weight were recorded daily. Other anthropometric measurements (length, skin folds, and head circumference) were recorded weekly. Weight was measured on digital scales with a 1 gm precision, length was measured with a Lucite length board with a precision of 1 mm, and head circumference was measured with a nonstretching tape with a precision of 2 mm. On the basis of a comparison of observed anthropometric values after the postconceptional age of 40 weeks with the National Center

for Health Statistics reference standard, *z* scores were calculated according to the following equation^{33,34}

$$z \text{ Score} = \frac{\text{Observed value} - \text{Median value for age and sex}}{\text{SD for a given age and sex}}$$

Subcutaneous fat folds were assessed by means of Lange calipers with a precision of 0.2 mm. During the follow-up to the adjusted postnatal age of 4 months, infants continued receiving the same dietary EFA regimen and were assessed monthly by a physician investigator and a research nurse. A total of 31 infants (37%) dropped out of the study during the follow-up phase. Twenty mothers did not return to the clinic for follow-up, one infant (receiving corn oil-based formula A) died of sudden infant death syndrome, one infant was adopted by parents who chose not to continue participation in the study, the families of six infants moved from the city, and gastrointestinal intolerance developed in one infant in each formula group. Dropouts from each group by 57 weeks of postconceptional age were 1 of 10 from the human milk group, 7 of 20 in the corn oil group (formula A), 9 of 25 in the soy oil group (formula B), and 14 of 28 in the soy oil-marine oil group (formula C).

Increased bleeding time is a potentially adverse effect of feeding ω 3 fatty acids. We therefore measured the bleeding times with a spring-activated device (Surgicutt; International Technidyne Corp., Edison, N.J.) for use with newborn infants.^{35,36} This device produced a cut 0.5 mm deep and 5 mm long, perpendicular to the main axis of the forearm, and 2 cm below the antecubital area. For comparative purposes, an adult device was used in the opposite arm to produce a cut 1.0 mm deep and 5 mm long. On day 10 only the pediatric device was used to avoid unnecessary stress. A blood pressure cuff was applied to the upper arm to maintain a pressure of 25 mm Hg while bleeding time was measured. Filter paper was used to blot the area every 30 seconds. Time required for bleeding to stop was recorded to the nearest half minute. Platelets were counted with a phase-contrast microscope at the time of the bleeding studies.

Measurements of vitamins A and E. Vitamin A and vitamin E were analyzed by high-pressure liquid chromatography according to the procedure of Catignani and Bieri³⁷ as modified by Ibarra et al.³⁸ Vitamin A acetate was added as an internal standard. Plasma lipids were extracted with hexane and suspended in ethanol. Lipids were fractionated on a 25 cm octadecyl-silica-18 reverse-phase analytic column with the use of a series 4 liquid chromatograph (Perkin-Elmer Corp., Norwalk, Conn.). Retinyl acetate (internal standard) (retention time = 6.5 min), vitamin A (retention time = 5.9 min), and vitamin E (retention time = 7.3 min) were detected at 300 nm. Data were obtained at 57 weeks and expressed as micrograms of vita-

min A per deciliter and micrograms of vitamin E per milliliter of plasma.

Membrane fluidity. Erythrocyte membrane fluidity was evaluated in duplicate samples of fresh whole blood drawn from study infants. Erythrocytes were washed three times in isotonic buffer, adjusted to pH 7.4, and taken to a hematocrit value of 0.2% cell volume; steady-state fluorescence of diphenylhexatriene was determined according to the method of Eisinger and Flores.³⁹ The polarization studies were conducted at 25° and 37° C in a manner similar to that which we have previously described for cultured retinal cells.⁴⁰ Briefly, diphenylhexatriene was added to the diluted erythrocyte suspension and incubated in the dark for 30 minutes, and fluorescent excitation was accomplished with a filtered and attenuated xenon lamp beam set at 364 nm. The sample, in a quartz microcuvette, was placed in a thermostated cell holder of a custom-built T-format polarization photometer equipped with Glan-Taylor calcite polarizers. The emission was detected with high-sensitivity photomultiplier tubes and photon-counting electronics. Polarization (P) was calculated according to the following equation⁴¹:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

where I_{\parallel} and I_{\perp} are the intensity outputs of the two photon counters corresponding to the parallel and perpendicular polarized components of the emission. Polarization, as a measure of the structural ordering of the membrane lipids, is inversely proportional to rotational fluidity in the membrane environment adjacent to the fluoroprobe and is reported in millipolarization units (polarization units $\times 10^3$).

Statistics and sample size. A sample size of 12 per group would permit detection of an absolute difference of 4% in the mean value of a specific fatty acid fraction with a 0.05 level of significance (α error) and a power of 0.9 ($1-\beta$ error) (provided the variance for the group mean was $<8\%$).⁴² We selected fatty acid compositions to estimate sample size because we had preliminary data of the group variance for this measurement. However, we also considered that a sample of 15 subjects would permit detection of differences of more than 1.2 SD for the other variables of interest (growth, bleeding time, erythrocyte fluidity, vitamin levels) with an $\alpha = 0.05$ and a power of 0.8. We planned to include 20 to 25 neonates in each formula group to ensure that 15 or more would complete the outpatient follow-up. Because the dropout rate was high after discharge, we analyzed data from those who had complete anthropometry, dietary intake, bleeding time, and fluidity data through 40 weeks ($n = 70$). In addition, a separate statistical analysis using

Table 1. Study infant growth data

	Formula group			
	Human milk group*	Corn oil (A)	Soy oil (B)	Soy oil and marine oil (C)
Gestation (wk)†	30.2 ± 1.2	30.3 ± 1.8	29.9 ± 1.5	30.4 ± 1.6
Gender (F/M) entry to 40 wk†	5/5	10/8	12/8	13/9
Gender (F/M), 40 to 57 wk‡	5/4	6/7	11/5	9/5
Body weight, birth (gm)†	1309 ± 117	1325 ± 121	1281 ± 121	1296 ± 112
Entry weight (gm)†	1212 ± 86	1275 ± 162	1257 ± 181	1218 ± 117
Weight, 34 wk (gm)†	1381 ± 108	1521 ± 208	1494 ± 223	1480 ± 153
Weight, 40 wk (gm)†	2890 ± 431	3251 ± 413	3035 ± 413	3170 ± 478
Weight, 48 wk (gm)‡	4729 ± 635	4688 ± 459	4637 ± 578	5112 ± 487
Weight, 57 wk (gm)‡	6114 ± 779	5875 ± 540	6045 ± 753	6453 ± 719
Days to 1800 gm†	34.8 ± 5.7	29.4 ± 6.1	31.5 ± 8.1	30.0 ± 6.4
Body length, birth (cm)†	39.2 ± 1.8	39.3 ± 1.5	39.0 ± 1.8	39.0 ± 1.6
Length, 34 wk (cm)†	40.4 ± 1.4	40.4 ± 1.7	40.2 ± 2.1	40.2 ± 1.5
Length, 40 wk (cm)†	47.8 ± 2.5	48.5 ± 2.0	48.5 ± 2.2	48.2 ± 1.9
Length, 48 wk (cm)‡	54.8 ± 1.4	55.4 ± 1.9	55.1 ± 2.0	55.3 ± 2.5
Length, 57 wk (cm)‡	61.0 ± 1.9	60.5 ± 2.1	60.7 ± 2.4	61.2 ± 2.3
Head circumference, birth (cm)†	27.5 ± 0.9	27.5 ± 1.2	27.3 ± 1.4	27.2 ± 1.3
Head circumference, 34 wk (cm)†	28.6 ± 0.9	29.0 ± 1.3	28.7 ± 1.5	28.7 ± 1.3
Head circumference, 40 wk (cm)†	34.9 ± 1.5	35.2 ± 1.0	34.8 ± 1.3	35.2 ± 1.1
Head circumference, 48 wk (cm)‡	38.9 ± 0.9	39.1 ± 1.3	38.7 ± 1.4	39.5 ± 1.2
Head circumference, 57 wk (cm)‡	41.0 ± 0.9	41.4 ± 1.3	41.2 ± 1.3	41.6 ± 1.4
Triceps fat, 34 wk (mm)†	2.9 ± 0.8	3.2 ± 0.6	3.0 ± 0.9	3.0 ± 0.6
Triceps fat, 40 wk (mm)†	6.3 ± 2.2	6.8 ± 1.6	6.1 ± 1.6	6.8 ± 1.9
Triceps fat, 57 wk (mm)‡	8.0 ± 1.8	8.7 ± 1.3	8.6 ± 1.7	9.3 ± 2.7
Subscapular fat, 34 wk (mm)†	3.1 ± 0.8	3.3 ± 0.7	2.7 ± 0.6	2.8 ± 0.9
Subscapular fat, 40 wk (mm)†	5.7 ± 1.6	7.2 ± 1.5	6.8 ± 1.5	6.6 ± 1.9
Subscapular fat, 57 wk (mm)‡	8.5 ± 1.6	7.9 ± 0.8	8.4 ± 2.0	8.3 ± 1.7
Kcal intake/kg, wk 1†	90 ± 26	88 ± 15	85 ± 15	95 ± 16
Kcal intake/kg, wk 2†	102 ± 22	103 ± 11	97 ± 17	102 ± 18
Kcal intake/kg, wk 3†	105 ± 29	106 ± 16	105 ± 13	108 ± 19
n with HMD†	5	10	8	14

The 40-, 48-, and 57-week time points correspond to outpatient follow-up visits. Diet effects were tested by ANOVA; no statistical differences between groups ($p < 0.05$) were found.

HMD, Hyaline membrane disease.

*The human milk group was not chosen by randomization. For details, see text.

†Values (mean ± SD) correspond to subjects with complete data at 40 weeks of postconceptional age ($n = 70$). Number of infants in each diet group: human milk 10; formula A, 18; formula B, 20; formula C, 22.

‡Values (mean ± SD) correspond to subjects completing 57-week follow-up ($n = 52$). Number of infants in each diet group; human milk, 9; formula A, 13; formula B, 16; formula C, 14.

repeated-measures analysis of variance was conducted for those who completed the study through 57 weeks ($n = 52$). Conclusions on growth were based solely on the latter complete data set. Because the group fed human milk was selected by maternal preference and thus not randomly selected, this group was excluded from statistical comparisons with the randomly assigned formula groups.

Random assignment of infants into the low $\omega 3$ fatty acid corn oil–based formula A was interrupted after enrollment of 20 subjects because an interim analysis demonstrated significant differences in retinal function.²² The institutional review board decided that the evidence was conclusive and that continued enrollment of infants into the corn oil formula group was unwarranted because it might contribute to developmental delays.

Results of the effect of diet ($\omega 3$ fatty acid supply) and

postnatal age for the complete data set were analyzed by repeated-measures ANOVA. Intervening variables were controlled by patient-selection procedures and by the standardization of experimental conditions. Statistical analyses using ANOVA were conducted to ensure that groups were comparable in terms of the intervening variables. One-way ANOVA was used to analyze the effects at single time points. Repeated-measures ANOVA was used to compare the effect of diet and postnatal age on growth during follow-up. Multiple post hoc comparison of anthropometric and other results were analyzed by means of the Dunn test ($\alpha = 0.05$).⁴³

RESULTS

The characteristics of subjects ($n = 70$) on entry into the study and the caloric intake for the first 3 weeks of study are

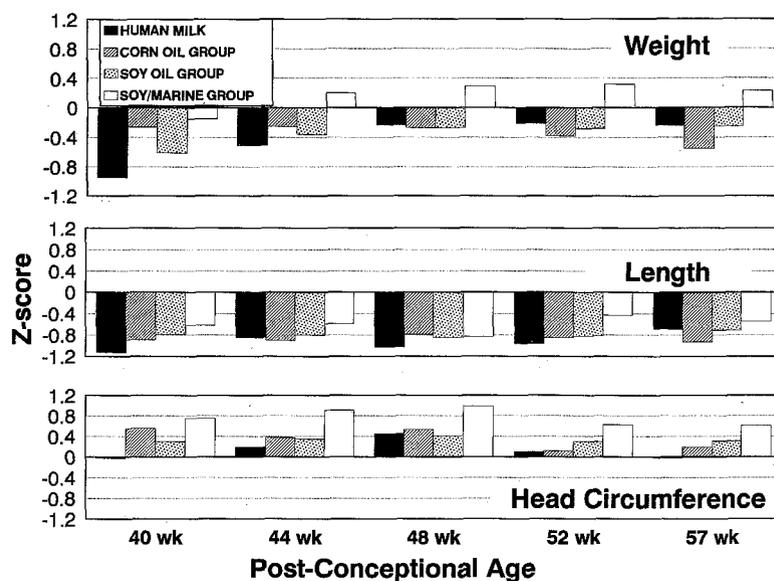


Figure. Standardized growth indexes for infants who completed the study ($n = 52$). Anthropometric measurements were transformed to z scores with the use of National Center for Health Statistics norms. Data for 40, 44, 48, 52, and 57 weeks correspond to $n = 52, 52, 50, 48,$ and $52,$ respectively. ANOVA demonstrated significant time effects but no diet-induced effects on growth.

shown in Table I. Subjects in the different groups had comparable birth weight, length, head circumference, gender, gestational age, weight at study entry, and gross energy intake. The prevalence of neonatal morbidity, including hyaline membrane disease, was similar among diet groups. Weight at 34 weeks of postconceptional age was included in Table I because some infants were discharged as early as 35 weeks. Weight, length, and head circumference of the infants did not show significant effects of diet. This result was supported further by results of standardized z scores for the 52 subjects who completed the study. In this subgroup the absolute growth data from birth to 40 weeks did not differ from values shown in Table I. Correlations between changes in standardized growth and relative 20:4 ω 6 or 22:6 ω 3 content in plasma and erythrocyte lipids at 57 weeks were made. The length z score at 57 weeks was negatively correlated with 20:4 ω 6 in total erythrocyte lipids at 57 weeks ($n = 52$; $r = -0.37$; $p = 0.008$). Significant age-dependent increases in weight, length, and head circumference were evident in all diet groups. None of the formula groups differed in triceps or subscapular skin-fold thickness. In summary, the data did not show evidence of adverse effects of ω 3 fatty acid supplementation on the growth of these VLBW infants. Furthermore, all groups grew well; mean values at 4 months of adjusted age were at or close to the 25th percentile for gestational age-corrected National Center for Health Statistics norms (Figure).

All bleeding time values were well within acceptable ranges (Table II).^{35,44} Bleeding time determinations with

the pediatric device at week 34 indicated a higher value in formula group C (consuming ω 3 LCPUFAs), but no value in that group exceeded the upper normal limit (7 minutes). The maximal values observed at 34 weeks, including all groups, were 3 minutes with the pediatric device and 4 minutes with the adult device; at 4 months the maximal values were 2½ and 5 minutes, respectively. Values obtained with the adult bleeding time device were always higher than those obtained with the pediatric device. Platelet counts done concomitantly with the bleeding time measurements were all within normal limits.

We found no diet-related changes in the rotational membrane fluidity of intact erythrocytes from infants on the four diets with the use of diphenylhexatriene fluorescence polarization,⁴⁵ as shown in Table III. However, a significant decrease was noted between ages for combined diet groups at both temperatures ($p < 0.05$). In addition, plasma values (mean \pm SD) of vitamin A (in micrograms per deciliter) and vitamin E (in micrograms per milliliter) measured at the 57-week follow-up were within normal limits. The respective values for infants in the corn oil group (formula A) were 33.0 ± 9.9 and 11.9 ± 3.5 ; for the soy oil group (formula B), 34.9 ± 7.9 and 10.8 ± 2.5 ; for the soy oil-marine oil group (formula C), 35.1 ± 8.9 and 15.4 ± 3.9 ; and for the group fed human milk, 33.0 ± 11.0 and 13.9 ± 4.8 . No significant diet-induced differences in vitamin A or E values were found in the study group infants. Values were not obtained at earlier time points because blood sample size was limiting.

Table II. Infant bleeding time results

	Human milk group*	Formula group		
		Corn oil (A)	Soy oil (B)	Soy oil and marine oil (C)
Entry, pediatric device (min)	2.00 ± 0.58	1.73 ± 0.65	1.85 ± 0.61	2.20 ± 0.52
34 wk, pediatric device (min)	1.60 ± 0.61	1.67 ± 0.52 ^a	1.68 ± 0.66 ^{a, b}	2.15 ± 0.69 ^b
34 wk, adult device (min)	3.14 ± 1.21	3.46 ± 0.86	3.40 ± 1.43	3.25 ± 0.77
57 wk, pediatric device (min)	1.93 ± 0.53	1.36 ± 0.75	1.25 ± 0.69	1.25 ± 0.71
57 wk, adult device (min)	5.17 ± 2.07	4.63 ± 2.39	3.88 ± 1.11	3.17 ± 1.17

All values are mean ± SD. Diet effects were tested by ANOVA ($F = 3.94$; $p < 0.012$). Group differences were tested by the Dunn t test.

*The human milk group was not chosen by randomization. For details, see text.

^{a, b}Values with different superscripts are significantly different ($p < 0.05$).

Table III. Membrane fluidity of study infant erythrocyte membranes

	Human milk group*	Formula group		
		Corn oil (A)	Soy oil (B)	Soy oil and marine oil (C)
Entry at 25° C (mP)	235 ± 34	216 ± 32	209 ± 24	218 ± 34
36 wk at 25° C (mP)	221 ± 32	210 ± 30	202 ± 30	211 ± 30
57 wk at 25° C (mP)	222 ± 31	203 ± 24	207 ± 22	208 ± 25
Entry at 37° C (mP)	206 ± 41	185 ± 33	177 ± 23	189 ± 34
36 wk at 37° C (mP)	192 ± 30	177 ± 31	172 ± 28	184 ± 29
57 wk at 37° C (mP)	186 ± 30	172 ± 19	178 ± 22	180 ± 27

All values are mean ± SD. Diet effects were tested by ANOVA.

mP, Millipolarization units ($P \times 10^3$).

*The human milk group was not chosen by randomization. For details, see text.

DISCUSSION

Our study was designed to assess the efficacy and safety of $\omega 3$ fatty acid supplementation for premature infants with the use of soy oil or soy oil–marine oil combinations. Subject selection was restricted to the healthiest VLBW infants, and sample size was chosen to detect fairly large differences. Thus the results for safety and efficacy should be interpreted in this context.

To evaluate efficacy, we used clinical electrophysiology and psychophysics. Full-field rod and cone electroretinograms were used to measure retinal effects. Visual acuity was determined electrophysiologically by pattern-reversal visual-evoked potentials to evaluate cortical effects and psychophysically by forced-choice preferential looking to evaluate visual behavior. The results of these and other studies indicated that the $\omega 3$ fatty acid supply of the early diet was related to visual maturation.* The reversibility of decreased visual function caused by 18:3 $\omega 3$ deficiency was uncertain because our study was terminated at 57 weeks of postconceptional age. However, long-term follow-up data from primate studies suggest that vision abnormalities do

not recover fully after dietary replenishment with $\omega 3$ fatty acids.^{47, 48}

The safety aspects of the evaluation included measurements of growth, bleeding function, erythrocyte membrane properties, and vitamin A and E levels. In addition, studies of peroxidation of erythrocyte membrane lipids and erythrocyte membrane fragility demonstrated no significant differences among the infant groups fed human milk and those fed formula.²⁵ The only potential biochemical anomaly detected in the marine oil-supplemented group was a 12% to 33% reduction in 20:4 $\omega 6$ content of erythrocyte lipids, which may be attributable to a high content of eicosapentaenoic acid (20:5 $\omega 3$) in the formula, that is, 0.6% of total fatty acids (see article by Hoffman and Uauy²⁵ for details). Regardless of this result, no adverse effects of marine oil supplementation were evident; all values (including growth) were within the acceptable ranges by clinical standards of care for VLBW infants. The analysis of anthropometric data by means of absolute values or standardized z scores revealed no significant differences among diet groups. One other study has reported the results of marine oil supplementation for preterm infants, but direct comparison with our study is difficult because of a difference in experimental

*References 14-16, 22, 26, 27, 31, 46-48.

design.^{31, 49, 50} In that study, long-term (92 weeks after conception) feeding with a marine oil-supplemented formula was associated with decreased growth and a low 20:4 ω 6 concentration in erythrocyte lipids. This relationship is in contrast to the negative correlation between length z score and erythrocyte 20:4 ω 6 found in our shorter-duration (57 weeks after conception) study. These differences could also be attributable to inclusion criteria of the Carlson study,^{49, 50} which permitted a sicker population of preterm infants as defined by birth weight, ventilator hours, and onset of enteral feedings. Further differences between the studies may also be associated with different concentrations of 18:2 ω 6 or micronutrients, or both, used in the follow-up formulas. Additional and larger trials are required to establish the safest mechanism and duration for providing these essential nutrients to infants.

Alternative sources of specific ω 3 fatty acids are presently under investigation. Recently the European Society for Pediatric Gastroenterology and Nutrition⁵¹ recommended that VLBW infant formula be supplemented with long-chain ω 3 and ω 6 fatty acids. The results published here and elsewhere^{21, 24-26} support that recommendation with respect to ω 3 fatty acid. During 1992, two formulas containing ω 3 LCPUFA were commercially released in Germany and Spain. The impact of these formulas on the EFA status of preterm infants has been assessed only in short-term studies, and the assessments have not included a functional measure such as visual development. The American Academy of Pediatrics Committee on Nutrition has not yet recognized dietary ω 3 fatty acids as essential for VLBW infants; thus no recommendations exist for their inclusion in formula in the United States. Our findings suggest that dietary supplementation of ω 3 EFA does not impose a safety risk for VLBW infants.

Bleeding time measurements were conducted with the expert advice of Dr. George Buchanan, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas. Chromatography of vitamins was completed with the assistance of Dr. J. C. Argyle, Pediatric Pathology, Children's Medical Center, University of Texas Southwestern Medical Center.

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