

## ORIGINAL ARTICLE

# Docosahexaenoic Acid and Bronchopulmonary Dysplasia in Preterm Infants

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## ABSTRACT

**BACKGROUND**

Studies in animals and in humans have suggested that docosahexaenoic acid (DHA), an n-3 long-chain polyunsaturated fatty acid, might reduce the risk of bronchopulmonary dysplasia, but appropriately designed trials are lacking.

**METHODS**

We randomly assigned 1273 infants born before 29 weeks of gestation (stratified according to sex, gestational age [ $<27$  weeks or 27 to  $<29$  weeks], and center) within 3 days after their first enteral feeding to receive either an enteral emulsion providing DHA at a dose of 60 mg per kilogram of body weight per day or a control (soy) emulsion without DHA until 36 weeks of postmenstrual age. The primary outcome was bronchopulmonary dysplasia, defined on a physiological basis (with the use of oxygen-saturation monitoring in selected infants), at 36 weeks of postmenstrual age or discharge home, whichever occurred first.

**RESULTS**

A total of 1205 infants survived to the primary outcome assessment. Of the 592 infants assigned to the DHA group, 291 (49.1% by multiple imputation) were classified as having physiological bronchopulmonary dysplasia, as compared with 269 (43.9%) of the 613 infants assigned to the control group (relative risk adjusted for randomization strata, 1.13; 95% confidence interval [CI], 1.02 to 1.25;  $P=0.02$ ). The composite outcome of physiological bronchopulmonary dysplasia or death before 36 weeks of postmenstrual age occurred in 52.3% of the infants in the DHA group and in 46.4% of the infants in the control group (adjusted relative risk, 1.11; 95% CI, 1.00 to 1.23;  $P=0.045$ ). There were no significant differences between the two groups in the rates of death or any other neonatal illnesses. Bronchopulmonary dysplasia based on a clinical definition occurred in 53.2% of the infants in the DHA group and in 49.7% of the infants in the control group ( $P=0.06$ ).

**CONCLUSIONS**

Enteral DHA supplementation at a dose of 60 mg per kilogram per day did not result in a lower risk of physiological bronchopulmonary dysplasia than a control emulsion among preterm infants born before 29 weeks of gestation and may have resulted in a greater risk. (Funded by the Australian National Health and Medical Research Council and others; Australian New Zealand Clinical Trials Registry number, ACTRN12612000503820.)

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**B**RONCHOPULMONARY DYSPLASIA IS A SERIOUS complication of preterm birth. It is characterized by an inflammatory process causing abnormal lung development, decreased vascular and alveolar development,<sup>1</sup> and the need for supplemental oxygen or assisted ventilation at 36 weeks of postmenstrual age (gestational age [weeks between the first day of the last menstrual period and birth] plus chronological age [weeks elapsed after birth]).<sup>2</sup> Bronchopulmonary dysplasia is associated with long-term pulmonary and neurodevelopmental impairment and increased needs for health and education services.<sup>3,4</sup>

Some evidence has suggested that the n-3 long-chain polyunsaturated fatty acid docosahexaenoic acid (DHA) may help protect against the development of bronchopulmonary dysplasia. Enriching diets with DHA can change the inflammatory balance, modulating cell function and suppressing inflammatory responses.<sup>5,6</sup> Among prespecified secondary outcomes in our previous randomized, controlled trial, which was designed to assess the effect of DHA on developmental outcomes,<sup>7</sup> we observed a lower incidence of bronchopulmonary dysplasia with a DHA-supplemented diet than with a control diet in the subgroup of infants who had a birth weight of less than 1250 g ( $P=0.04$ ).<sup>8</sup> Observational data from infants born before 30 weeks of gestation show an association between low blood levels of DHA and bronchopulmonary dysplasia.<sup>9</sup> In addition, studies in animals have shown reduced inflammatory responses, improved lung growth, and increased production of pulmonary surfactant with DHA supplementation.<sup>10-14</sup> In preterm infants, direct enteral DHA supplementation from the onset of feeding is associated with an acceptable side-effect profile and inhibits the early decline in DHA levels that is typically seen during the transition to full enteral feeding.<sup>15</sup> We conducted this randomized trial to determine the effect of DHA supplementation on the incidence of bronchopulmonary dysplasia among infants born before 29 weeks of gestation.

## METHODS

### TRIAL DESIGN AND OVERSIGHT

The N-3 Fatty Acids for Improvement in Respiratory Outcomes (N3RO) trial, designed and conducted by the authors, was a randomized, blinded, controlled trial that was conducted at 13 centers in Australia, New Zealand, and Singa-

pore. The human research ethics committee responsible for each participating center approved the trial protocol<sup>16</sup> (available with the full text of this article at NEJM.org), and appropriate regulatory approvals were obtained. (For details, see the Supplementary Appendix, available at NEJM.org.) The protocol was peer reviewed by the IMPACT Clinical Trials Network for Mothers' and Babies' Health. An independent serious-adverse-event committee evaluated deaths. An independent trial monitoring committee reviewed trial safety and progress. The committee had access to key trial outcomes according to group, including the primary outcome. No formal interim analysis of efficacy was planned or undertaken; the committee performed two reviews of safety and recommended that the trial continue. The Australian National Health and Medical Research Council funded the trial, and Clover (Westmeadows, VIC, Australia) donated the trial product; these entities had no role in the design or conduct of the trial; the collection, analysis, or interpretation of the data; the writing, review, or approval of the manuscript; or the decision to submit the manuscript for publication. The first four authors and the last author had full access to all the data and vouch for the completeness and accuracy of the data. All the authors vouch for the fidelity of the trial to the protocol.

### PARTICIPANTS

Infants born before 29 weeks of gestation who had commenced enteral feeding in the previous 3 days were eligible to participate. Infants were ineligible if they had a major congenital or chromosomal abnormality, were participating in another trial of fatty-acid supplementation, or were receiving intravenous lipids containing fish oil or if the mother wanted to provide breast milk and take supplements containing DHA at a dose of more than 250 mg per day.

### RANDOMIZATION AND BLINDING

A computer-generated randomization schedule that used balanced variable blocks of 2, 4, and 6 in a ratio of 1:2:1 was prepared by an independent statistician. Randomization was stratified according to sex, gestational age (<27 completed weeks or 27 to <29 weeks), and center. Infants from multiple births underwent randomization individually. Research nurses assessed infants for eligibility and invited the parents or guardians of eligible infants to enter their infant in the

trial. After written informed consent was obtained, infants were randomly assigned with the use of our Web-based randomization service. A unique trial identification number was assigned to each infant, and a unique product identification number identified the trial emulsion containing the intervention or control emulsion, prepackaged according to the randomization schedule. Participants, their families, and clinical and research personnel were unaware of the randomization assignments. The intervention and control emulsions were identical in viscosity, color, packaging, and labeling.

#### TRIAL INTERVENTION

Both the DHA emulsion and the soy emulsion were emulsified with 1% lecithin and contained 20% total fat and 6% protein. The DHA emulsion comprised a microencapsulated aqueous emulsion of fractionated tuna oil (70% of total oil as DHA in triglyceride form); 0.5 ml of the DHA emulsion contained 60 mg of DHA, 4 mg of eicosapentaenoic acid, 4.6 mg of vitamin C, and 0.05 mg of vitamin E. For the control emulsion, 0.5 ml contained 55 mg of linoleic acid, 5.7 mg of alpha-linolenic acid, 4.8 mg of vitamin C, and 0.01 mg of vitamin E.

The emulsions were administered enterally, at a dose of 0.17 ml per kilogram of body weight three times daily (total, 0.5 ml per kilogram per day). Thus, infants in the DHA group received 60 mg of DHA per kilogram per day, and those in the control group received no additional DHA. The treatment dose provided sufficient DHA to match the estimated fetal accretion rate during the last trimester.<sup>17</sup> The dose calculation was adjusted weekly for changes in body weight. The trial emulsions were given immediately before a feeding through a nasogastric or orogastric tube; if feedings were stopped, the emulsion was withheld and was recommenced as soon as feedings restarted. The trial emulsion was continued until the primary outcome assessment. The emulsion met quality thresholds throughout and beyond the stated product shelf life.<sup>18</sup> Apart from the administration of the trial emulsion, clinical management was under the direction of neonatologists according to local protocols. The use of intravenous lipid emulsions containing fish oil for the treatment of liver disease associated with parenteral nutrition was at the discretion of the attending neonatologist, with the trial emulsions continued when enteral feedings occurred.

#### OUTCOMES

The primary outcome was the incidence of physiological bronchopulmonary dysplasia (assessed at 36 weeks of postmenstrual age or discharge home, whichever occurred first), as determined on the basis of requirements for supplemental oxygen or respiratory support with an assessment of oxygen saturation.<sup>16,19</sup> Secondary respiratory outcomes included the composite of physiological bronchopulmonary dysplasia or death before 36 weeks of postmenstrual age, clinical bronchopulmonary dysplasia (defined by clinical management with supplemental oxygen or respiratory support at the time of the primary outcome assessment), severity of bronchopulmonary dysplasia (according to National Institute of Child Health and Human Development [NICHD] definitions<sup>2,19</sup>), and the requirement for respiratory support. Other secondary outcomes included retinopathy of prematurity, intraventricular hemorrhage, sepsis, necrotizing enterocolitis, and measures of safety and tolerance (death before 36 weeks of postmenstrual age and during the first hospitalization, length of hospital stay, feeding tolerance [number of days to reach full enteral feeding and number of days on which one or more feedings were stopped], and growth). (For definitions of outcomes, see the Supplementary Appendix.) All the infants were followed to the expected date of delivery or first discharge home, whichever occurred first. A whole-blood specimen (30  $\mu$ l) was obtained at randomization and at the primary outcome assessment for fatty-acid analysis.<sup>20</sup>

#### STATISTICAL ANALYSIS

On the basis of an estimated incidence of bronchopulmonary dysplasia among infants born before 29 weeks of gestation of 51.4%,<sup>7</sup> we calculated that 1244 infants (622 per group) would need to be enrolled for the trial to have 90% power to detect an absolute difference of 10 percentage points and a relative difference of 19% between trial groups in the incidence of bronchopulmonary dysplasia at 36 weeks of postmenstrual age, at a two-tailed alpha level of 0.05. The sample accounts for a variance inflation factor of 15% (11% multiple births and 4% death rate before 36 weeks of postmenstrual age, as estimated from our previous trial<sup>7</sup>). Analyses were performed on an intention-to-treat basis according to a prespecified statistical analysis plan. The incidence of bronchopulmonary dysplasia was

compared between groups with the use of a log-binomial regression model, with a generalized estimating equation used to account for clustering of outcomes due to multiple births. Adjustment was made for randomization strata. Secondary analyses used log-binomial, linear, and negative-binomial regression models with generalized estimating equations for binary, continuous, and count outcomes, respectively.

Missing data (excluding deaths) on physiological bronchopulmonary dysplasia, mild bronchopulmonary dysplasia, moderate bronchopulmonary dysplasia, days on which one or more feedings were stopped, and growth were multiply imputed according to treatment group with the use of chained equations. (For further details, see the Supplementary Appendix.) Multiple imputation was not applied for other outcomes, given the completeness of data (<1% missing data).

In planned subgroup analyses (76 total), we examined the effect of DHA supplementation according to the randomization strata of sex and gestational age; up to four significant interaction tests ( $P<0.05$ ) were expected owing to chance alone. All analyses were performed with the use of SAS software, version 9.3 (SAS Institute), with blinding to treatment group.

## RESULTS

### TRIAL PARTICIPANTS

From June 18, 2012, to September 30, 2015, a total of 1098 women with 1273 infants underwent randomization; 631 infants were assigned to receive the DHA emulsion and 642 to receive the control emulsion (Fig. 1). (For the number of infants recruited at each center, see the Supplementary Appendix.) One infant in the DHA group was determined after randomization to have been born after 29 weeks of gestation and remained in the intention-to-treat analysis. Primary outcome data were available for 1149 infants (90.3%); 68 infants (5.3%) died before 36 weeks of postmenstrual age and 56 (4.4%) were missing data on the primary outcome (Fig. 1). After imputation (excluding deaths), 1205 infants (592 in the DHA group and 613 in the control group) were included in the primary outcome analysis.

The baseline characteristics of the infants and their mothers were similar in the two treatment groups (Table 1, and Table S1 in the Supplementary Appendix). A mean of 90% of ordered

### Figure 1 (facing page). Screening, Randomization, and Follow-up.

DHA denotes docosahexaenoic acid.

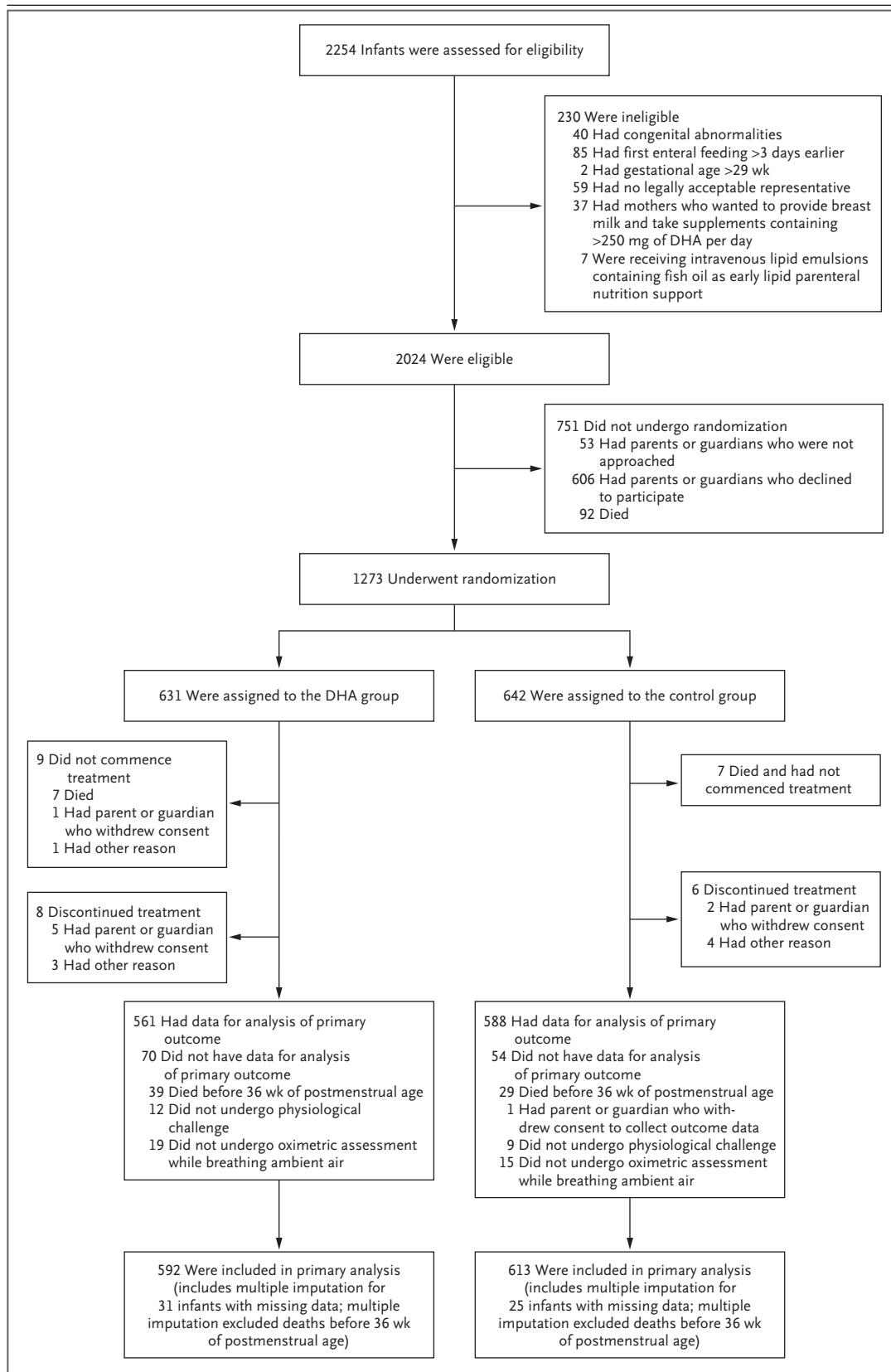
doses was given overall in each group. In the first week after randomization, adherence was lowest, because the trial emulsion could be given only with a feeding (mean [ $\pm$ SD] percentage of doses given,  $68\pm 25\%$  in the DHA group and  $69\pm 25\%$  in the control group). The baseline level of DHA in whole blood was  $2.7\pm 0.9\%$  of total fatty acids in each group (Table 1). At the time of the primary outcome assessment (36.1 $\pm$ 0.3 weeks of postmenstrual age in each group), the DHA level was  $3.9\pm 0.7\%$  of total fatty acids in the DHA group and  $2.5\pm 0.6\%$  of total fatty acids in the control group ( $P<0.001$ ); additional results with respect to fatty-acid levels in infants are presented in Tables S1, S2, and S3 in the Supplementary Appendix. Overall, 59 infants received intravenous lipids containing fish oil (Table S4 in the Supplementary Appendix).

### PRIMARY OUTCOME

Physiological bronchopulmonary dysplasia at 36 weeks of postmenstrual age or discharge home, whichever occurred first, occurred in 291 of 592 infants (49.1% by multiple imputation) in the DHA group and 269 of 613 infants (43.9%) in the control group (Table 2). The unadjusted relative risk was 1.12 (95% confidence interval [CI], 0.99 to 1.26;  $P=0.07$ ); the adjusted relative risk was 1.13 (95% CI, 1.02 to 1.25;  $P=0.02$ ).

### SECONDARY OUTCOMES

The composite outcome of physiological bronchopulmonary dysplasia or death before 36 weeks of postmenstrual age occurred in 52.3% of the infants in the DHA group and 46.4% of the infants in the control group (adjusted relative risk, 1.11; 95% CI, 1.00 to 1.23;  $P=0.045$ ) (Table 2). The incidence of clinical bronchopulmonary dysplasia was 53.2% and 49.7%, respectively (adjusted relative risk, 1.09; 95% CI, 1.00 to 1.18;  $P=0.06$ ). The incidence of mild bronchopulmonary dysplasia was significantly lower in the DHA group than in the control group, but there were no significant between-group differences in moderate or severe bronchopulmonary dysplasia (Table 2). There were no significant differences between



<b>Table 1. Baseline Characteristics of the Infants and Their Mothers.*</b>		
<b>Characteristic</b>	<b>DHA Group (N=631)</b>	<b>Control Group (N=642)</b>
<b>Mothers†</b>		
Age — yr‡	30.5±5.7	30.4±5.9
Race or ethnic group — no./total no. (%)§		
Australian Aboriginal, Torres Strait Islander, New Zealand Maori, or Native American	74/613 (12.1)	66/617 (10.7)
Asian	148/613 (24.1)	133/617 (21.6)
White	374/613 (61.0)	385/617 (62.4)
Black or Pacific Islander	17/613 (2.8)	33/617 (5.3)
Fish oil n-3 long-chain polyunsaturated fatty-acid supplements during pregnancy — no./total no. (%)	120/597 (20.1)	130/619 (21.0)
Smoked cigarettes during pregnancy — no./total no. (%)	107/608 (17.6)	104/619 (16.8)
Exposure to antenatal glucocorticoids — no./total no. (%)	586/631 (92.9)	592/641 (92.4)
Cesarean delivery — no. (%)	373 (59.1)	373 (58.1)
Histopathological evidence of chorioamnionitis or funisitis — no./total no. (%)	211/595 (35.5)	211/597 (35.3)
<b>Infants</b>		
Female sex — no. (%)	293 (46.4)	300 (46.7)
Gestational age <27 wk — no. (%)	322 (51.0)	317 (49.4)
Gestational age — wk	26.7±1.5	26.7±1.5
Singleton birth — no. (%)	450 (71.3)	461 (71.8)
Birth weight — g	913±236	924±239
Apgar score at 5 minutes — median (IQR)¶	8.0 (7.0–9.0)	8.0 (6.0–9.0)
Intubated in the delivery room — no./total no. (%)	386/630 (61.3)	388/642 (60.4)
Age at randomization — days		
Median	3.0	3.0
IQR	2.0–4.0	2.0–4.0
Age at first enteral feeding — days		
Median	2.0	2.0
IQR	1.0–3.0	1.0–3.0
DHA level in whole blood at randomization — % of total fatty acids	2.7±0.9	2.7±0.9
Age at first dose of trial emulsion — days**		
Median	4.0	4.0
IQR	3.0–5.0	3.0–5.0

\* Plus–minus values are means ±SD. There were no significant differences ( $P \geq 0.05$ ) between the docosahexaenoic acid (DHA) group and control group for baseline variables. IQR denotes interquartile range.

† Maternal characteristics have been summarized at the infant level (i.e., women were counted multiple times if they had multiple infants).

‡ Data were available for 626 mothers in the DHA group and 639 mothers in the control group.

§ Race and ethnic group were reported by the participants.

¶ Data were available for 629 infants in the DHA group and 637 infants in the control group.

|| Data were available for 626 infants in the DHA group and 635 infants in the control group.

\*\* Data were available for 622 infants in the DHA group and 635 infants in the control group.

**Table 2. Primary Outcome and Secondary Respiratory-Related Outcomes.\***

Outcome	DHA Group (N=592)	Control Group (N=613)	Adjusted Effect (95% CI)	Adjusted P Value
Physiological BPD: primary outcome — no. (%)†	291 (49.1)	269 (43.9)	1.13 (1.02–1.25)	0.02
Physiological BPD or death before 36 wk of postmenstrual age — no./total no. (%)†‡	330/631 (52.3)	298/642 (46.4)	1.11 (1.00–1.23)	0.045
Clinical BPD — no./total no. (%)	315/592 (53.2)	304/612 (49.7)	1.09 (1.00–1.18)	0.06
Severity of BPD				
Mild — no. (%)†§	80 (13.5)	108 (17.6)	0.76 (0.58–0.99)	0.04
Moderate — no. (%)†§	65 (11.0)	50 (8.1)	1.35 (0.95–1.92)	0.10
Severe — no./total no. (%)¶	202/592 (34.1)	194/612 (31.7)	1.07 (0.93–1.22)	0.36
Surfactant use — no./total no. (%)‡	533/631 (84.5)	516/642 (80.4)	1.05 (1.00–1.10)	0.06
Days of respiratory support**	41.5±28.7	40.4±27.7	1.02 (0.94–1.10)	0.63
Postnatal glucocorticoids — no./total no. (%)	128/604 (21.2)	132/622 (21.2)	0.98 (0.80–1.19)	0.81
Days of caffeine use††	61.9±19.3	60.7±18.9	1.01 (0.99–1.04)	0.29
Days of diuretic use††	4.5±13.2	5.2±13.7	0.70 (0.46–1.07)	0.10

\* Denominators exclude deaths before 36 weeks of postmenstrual age unless otherwise indicated. The relative risk or ratio of means (DHA group vs. control group) is reported as the effect. Values were adjusted for randomization strata: sex, gestational age (<27 weeks or 27 to <29 weeks), and center. Physiological bronchopulmonary dysplasia was determined on the basis of requirements for supplemental oxygen or respiratory support with an assessment of oxygen saturation. Clinical bronchopulmonary dysplasia was defined by clinical management with supplemental oxygen or respiratory support at the time of the primary outcome assessment.

† Missing data, excluding deaths, were multiply imputed. Average numerators across the 100 imputed data sets were rounded to the nearest integer value and hence may not correspond exactly with reported percentages.

‡ Denominators include deaths before 36 weeks of postmenstrual age.

§ The relative risk and P value were adjusted for country instead of center owing to the small number of events (<5) at some centers.

¶ Multiple imputation was not applied for severe bronchopulmonary dysplasia, given the completeness of data (<1% missing data).

|| Data are for any surfactant given, before or after randomization.

\*\* Shown are days of respiratory support until the assessment of the primary outcome at 36 weeks of postmenstrual age or discharge home, whichever occurred first. Data were available for 592 infants in the DHA group and 611 infants in the control group.

†† Data were available for 587 infants in the DHA group and 610 infants in the control group.

groups in the duration of respiratory support until the assessment of the primary outcome, postnatal glucocorticoid use, days receiving caffeine for lung disease, or days receiving diuretics for lung disease (Table 2).

There was no significant between-group difference in the risk of death before 36 weeks of postmenstrual age (6.2% in the DHA group and 4.5% in the control group; adjusted relative risk, 1.33; 95% CI, 0.84 to 2.12;  $P=0.23$ ) or in the percentage of infants who died before the first discharge home (7.9% in the DHA group and 5.9% in the control group; adjusted relative risk, 1.31; 95% CI, 0.88 to 1.96;  $P=0.19$ ) (Table 3). Nine deaths in the DHA group and eight in the control group were classified by the serious-adverse-event committee as resulting from bronchopul-

monary dysplasia. There were no significant differences between groups in the frequency of any major clinical outcome, including sepsis, retinopathy of prematurity, necrotizing enterocolitis, or intraventricular hemorrhage (Table 3, and Table S5 in the Supplementary Appendix). The number of days to reach full enteral feeding, the number of days on which one or more feedings were stopped, and the length of hospital stay were similar in the two groups (Table 3). The z scores for weight, length, and head circumference also did not differ significantly between groups at the time of the primary outcome assessment or at the expected date of delivery or discharge home, whichever occurred first (Table S6 in the Supplementary Appendix).

**Table 3. Secondary Clinical Outcomes.\***

Outcome	DHA Group (N=631)	Control Group (N=642)	Adjusted Effect (95% CI)	Adjusted P Value
Death before 36 wk of postmenstrual age — no. (%)	39 (6.2)	29 (4.5)	1.33 (0.84–2.12)	0.23
Death — no. (%)	50 (7.9)	38 (5.9)	1.31 (0.88–1.96)	0.19
Any intraventricular hemorrhage — no. (%)	207 (32.8)	184 (28.7)	1.11 (0.95–1.31)	0.19
Grade 3 or 4 intraventricular hemorrhage — no. (%)	44 (7.0)	33 (5.1)	1.32 (0.87–2.03)	0.20
Periventricular leukomalacia — no./total no. (%)†	8/592 (1.4)	8/612 (1.3)	1.01 (0.39–2.64)	0.98
Porencephalic cysts — no./total no. (%)†	7/592 (1.2)	10/612 (1.6)	0.71 (0.27–1.84)	0.48
Retinopathy of prematurity — no./total no. (%)†				
Unilateral, grade ≥3	15/589 (2.5)	12/612 (2.0)	1.29 (0.61–2.71)	0.51
Bilateral, grade ≥3	47/589 (8.0)	51/612 (8.3)	0.93 (0.64–1.33)	0.68
Requiring therapy	33/589 (5.6)	35/612 (5.7)	0.96 (0.61–1.50)	0.85
Sepsis — no./total no. (%)†	160/611 (26.2)	182/623 (29.2)	0.89 (0.76–1.05)	0.16
Necrotizing enterocolitis — no./total no. (%)†‡	50/601 (8.3)	44/619 (7.1)	1.16 (0.79–1.69)	0.46
Days to reach full enteral feeding§	17.8±20.1	17.9±13.3	0.99 (0.91–1.08)	0.78
No. of days on which ≥1 feeding was stopped¶	7.2±8.4	7.3±9.4	1.00 (0.87–1.14)	0.95
Length of hospital stay — days				
Median	91	87	0.91 (0.81–1.02)	0.09
IQR	72–112	71–112		

\* The relative risk or ratio of means (DHA group vs. control group) is reported as the effect, unless otherwise indicated. Values were adjusted for sex, gestational age (<27 weeks or 27 to <29 weeks), and either center (for any intraventricular hemorrhage, sepsis, and length of hospital stay) or country (for all other outcomes, owing to the small number of events [ $<5$ ] at some centers).

† Excluded were infants who did not have the condition and who died before 36 weeks of postmenstrual age.

‡ Data are for proven necrotizing enterocolitis defined according to the Australian and New Zealand Neonatal Network.<sup>21</sup>

§ Shown is the duration from birth until the first day that an enteral intake of at least 120 ml per kilogram of body weight per day was reached and was subsequently maintained for 3 consecutive days. Infants who did not reach full enteral feeding (29 infants in the DHA group and 19 in the control group) were excluded.

¶ Shown is the number of days on which one or more feedings were stopped until the assessment of the primary outcome. Missing data, excluding deaths and withdrawals, were multiply imputed.

|| The 88 infants who died were excluded. The treatment effect was calculated with the use of a subdistribution hazard ratio, with death treated as a competing risk. A time-varying coefficient was used for gestational age (<100 days or ≥100 days) to address nonproportionality.

#### SUBGROUP ANALYSES ACCORDING TO RANDOMIZATION STRATA

Primary and secondary outcomes stratified according to sex and gestational-age category are presented in Tables S7 through S10 in the Supplementary Appendix. There was no evidence of effect modification according to sex or gestational-age category for any outcome except days of diuretic use ( $P=0.02$  for interaction for both sex and gestational-age category); this finding should be interpreted with caution given the large number of interaction tests performed.

#### DISCUSSION

In this multicenter, randomized, controlled trial, an enteral emulsion providing 60 mg of DHA per kilogram per day was not associated with a

lower incidence of bronchopulmonary dysplasia (according to a physiological definition) than a control (soy) emulsion. The percentage of infants in whom bronchopulmonary dysplasia (defined physiologically) developed was higher with DHA supplementation than without supplementation (49.1% vs. 43.9%). When bronchopulmonary dysplasia was classified clinically according to the use of supplemental oxygen or respiratory support or limited to moderate or severe bronchopulmonary dysplasia, differences between groups were not significant.

The higher risk of the composite of physiological bronchopulmonary dysplasia or death before 36 weeks of postmenstrual age in the DHA group than in the control group was driven by the higher risk of bronchopulmonary dysplasia in the DHA group; the risk of death did not dif-

fer significantly between groups. The significantly lower incidence of NICHD-defined mild bronchopulmonary dysplasia in the DHA group than in the control group reflects a shift to more moderate or severe bronchopulmonary dysplasia in the infants who received DHA. We found no evidence of any other adverse effects overall or according to subgroup, including inflammatory-related neonatal illnesses (e.g., intraventricular hemorrhage, sepsis, and necrotizing enterocolitis). In contrast to smaller studies showing a decreased risk of retinopathy of prematurity associated with intravenous DHA supplementation,<sup>22-24</sup> our trial showed no such effect.

Our results highlight the importance of conducting adequately powered randomized trials to test new interventions.<sup>25</sup> Three previous trials that provided DHA at an amount estimated to meet fetal accretion rates and that examined outcomes with respect to bronchopulmonary dysplasia<sup>7,26</sup> or respiratory support<sup>27</sup> were underpowered for these outcomes. These previous trials (designed to determine the effect of DHA supplementation on development<sup>7,27</sup> and growth failure<sup>26</sup>) showed inconsistent effects, with one suggesting a reduction in bronchopulmonary dysplasia in infants with a birth weight of less than 1250 g,<sup>7,8</sup> and the others no effect on bronchopulmonary dysplasia<sup>26</sup> or the duration of respiratory support.<sup>27</sup> Our randomized trial was specifically designed to determine the effect of DHA on the risk of bronchopulmonary dysplasia and, despite biologic plausibility, showed no benefit.

We maximized the chance of detecting an effect of DHA on bronchopulmonary dysplasia with the mode of delivery (enteral emulsion) and DHA dose (60 mg per kilogram per day). DHA delivery in previous trials was through breast milk or formula,<sup>7,27,28</sup> and the full DHA dose could be achieved only when the infant reached full enteral feeding. By using direct enteral supplementation, we ensured that the full DHA dose was given independent of the amount of enteral feeding and that supplementation was commenced within the first days of life.<sup>15</sup> We used a DHA dose that was estimated to provide the fetal accretion rate of DHA.<sup>7,17</sup> In the context of background DHA content in breast milk and formula (providing a total of approximately 20 mg of DHA per kilogram per day when the infant was receiving full enteral feeding), the infants in our trial received a dose of DHA similar to that in the trial by Moltu et al.<sup>26</sup> (approximately 80 mg

of DHA per kilogram per day when the infant was receiving full enteral feeding); this dose was slightly higher than that used in other trials.<sup>7,27</sup> The current intervention resulted in blood DHA concentrations similar to those in our previous trial of DHA supplementation.<sup>29</sup>

The DHA preparation that we used did not include the n-6 fatty acid arachidonic acid, and blood levels of arachidonic acid at baseline and trial completion were similar to those observed in our previous trial.<sup>29</sup> There is controversy over the need for supplemental arachidonic acid.<sup>17,30</sup> Our earlier trial, which suggested a possible benefit of DHA in the prevention of bronchopulmonary dysplasia in infants with a birth weight of less than 1250 g,<sup>7,8</sup> also did not include arachidonic acid supplementation beyond that naturally occurring in breast milk and available in preterm infant formula. Supplementation with both DHA and arachidonic acid was used in previous trials that showed no significant differences between infants receiving supplementation and the control group with respect to bronchopulmonary dysplasia<sup>26</sup> or the requirement for respiratory support.<sup>27</sup>

We used a physiological definition of bronchopulmonary dysplasia that decreased variation in the diagnosis of the disorder among centers,<sup>19</sup> and we assessed the oxygen saturation of infants who were not receiving assisted ventilation and who were breathing ambient air at the time of the primary outcome assessment. Our prespecified definition of bronchopulmonary dysplasia included contemporary practices of supplemental oxygen that use high-flow devices and air delivered by high or low flow. We consider these results to be generalizable to preterm infants in high-income countries, which use similar treatment strategies. Bronchopulmonary dysplasia is difficult to define.<sup>2,31</sup> Extremely preterm infants can have impaired lung function irrespective of a diagnosis of bronchopulmonary dysplasia.<sup>32</sup> The complexity of defining the disorder highlights the difficulty in interpreting the clinical importance of our finding of a higher incidence of physiological bronchopulmonary dysplasia in the DHA group than in the control group. Longer term follow-up is important to assess the extent of airway disease in early childhood<sup>32,33</sup> as well as neurodevelopment.

Intravenous lipid emulsions providing DHA at doses similar to those given in our trial are being used to provide nutritional support during

the transition to full enteral feeding in preterm infants, although with limited testing in clinical trials.<sup>34,35</sup> Our results raise questions about the safety of this strategy and suggest the need for further study.

In conclusion, our results show that an enteral emulsion of DHA at a dose of 60 mg per kilogram per day did not result in a lower risk of bronchopulmonary dysplasia than control and may have resulted in a greater risk.

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#### APPENDIX

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