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High-Dose Docosahexaenoic Acid Supplementation of Preterm Infants: Respiratory and Allergy Outcomes

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KEY WORDS

docosahexaenoic acid, allergy, respiratory, premature infants

ABBREVIATIONS

BPD—bronchopulmonary dysplasia
DHA—docosahexaenoic acid
LCPUFA—long-chain polyunsaturated fatty acid
DINO—Docosahexaenoic Acid for the Improvement of Neurodevelopmental Outcome in Preterm Infants
AA—arachidonic acid
RR—relative risk
IQR—interquartile range
CI—confidence interval
DPPC—dipalmitoyl phosphatidylcholine

This trial has been registered with the Australian and New Zealand Clinical Trial Registry (anzctr.org.au) (identifier ACTRN12606000327583).

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FINANCIAL DISCLOSURE: *Dr Makrides serves on scientific advisory boards for Nestle, Fonterra, and Nutricia. Dr Gibson serves on scientific advisory boards for Nestle and Fonterra. Associated honoraria for Drs Makrides and Gibson are paid to their institutions to support conference travel and continuing education for postgraduate students and early career researchers. The other authors have indicated they have no financial relationships relevant to this article to disclose.*

WHAT'S KNOWN ON THIS SUBJECT: Very preterm infants are at risk of bronchopulmonary dysplasia and are more likely to suffer from atopic conditions in later life. Docosahexaenoic acid is known to modulate inflammation and is postulated to modulate the neonatal immune response.

WHAT THIS STUDY ADDS: High-dose docosahexaenoic acid supplementation may reduce the incidence of bronchopulmonary dysplasia in the smallest infants and male infants, and reduces reported hay fever at either 12 or 18 months in male infants but has no effect on other atopic conditions.

abstract

BACKGROUND: Docosahexaenoic acid (DHA) has been associated with downregulation of inflammatory responses.

OBJECTIVE: To report the effect of DHA supplementation on long-term atopic and respiratory outcomes in preterm infants.

METHODS: This study is a multicenter, randomized controlled trial comparing the outcomes for preterm infants <33 weeks' gestation who consumed expressed breast milk from mothers taking either tuna oil (high-DHA diet) or soy oil (standard-DHA) capsules. Data collected included incidence of bronchopulmonary dysplasia (BPD) and parental reporting of atopic conditions over the first 18 months of life.

RESULTS: Six hundred fifty-seven infants were enrolled (322 to high-DHA diet, 335 to standard), and 93.5% completed the 18-month follow-up. There was a reduction in BPD in boys (relative risk [RR]: 0.67 [95% confidence interval (CI): 0.47–0.96]; $P = .03$) and in all infants with a birth weight of <1250 g (RR: 0.75 [95% CI: 0.57–0.98]; $P = .04$). There was no effect on duration of respiratory support, admission length, or home oxygen requirement. There was a reduction in reported hay fever in all infants in the high-DHA group at either 12 or 18 months (RR: 0.41 [95% CI: 0.18–0.91]; $P = .03$) and at either 12 or 18 months in boys (RR: 0.15 [0.03–0.64]; $P = .01$). There was no effect on asthma, eczema, or food allergy.

CONCLUSIONS: DHA supplementation for infants of <33 weeks' gestation reduced the incidence of BPD in boys and in all infants with a birth weight of <1250 g and reduced the incidence of reported hay fever in boys at either 12 or 18 months. *Pediatrics* 2011;128:e71–e77

Very preterm infants are known to be at increased risk of long-term neurodevelopmental impairment compared with term infants.[1] The introduction of therapies such as antenatal corticosteroids and exogenous surfactant over the last 2 decades has improved respiratory and survival outcomes; however, respiratory distress syndrome and bronchopulmonary dysplasia (BPD) remain major causes of morbidity and mortality in the preterm infant. Studies also suggest that very preterm and very low birth weight infants are more likely to suffer morbidity from atopic conditions, including asthma, in later life.[2–5] The search continues for therapies that may improve outcomes in this high-risk population.

Docosahexaenoic acid (DHA) is an *n*-3 long-chain polyunsaturated fatty acid (LCPUFA), which along with the other *n*-3 LCPUFA, eicosapentaenoic acid, is provided in the diet by fish and fish oils. These LCPUFA are known to modulate inflammation and are also postulated to modulate the neonatal immune response. For example, supplementation with *n*-3 LCPUFA during pregnancy is known to be associated with decreased messenger RNA levels of TH2-related molecules in the fetus and decreased maternal inflammatory cytokines.[6] In pregnant atopic mothers, *n*-3 LCPUFA supplementation alters the infant cord blood hemopoietic phenotype, which may have an effect on the later development of atopic disease.[7] To the best of our knowledge, there are no randomized trials involving preterm infants that assess the effect of increasing the early dietary supply of *n*-3 LCPUFA on longer-term allergy outcomes.

The DINO (Docosahexaenoic Acid for the Improvement of Neurodevelopmental Outcome in Preterm Infants) trial[8] was originally designed to assess the effect of increasing dietary DHA during early life on the neurodevelopmental outcomes of preterm infants. We hypothesized that DHA would have a positive effect on allergic and respiratory outcomes due to the antiinflammatory compounds derived from DHA and eicosapentaenoic acid. We were able to determine if the effects of DHA intervention varied in the prespecified subgroups from DINO: birth weight and infant gender. This is the first report of the effect of DHA supplementation on long-term atopic and respiratory outcomes in preterm infants.

METHODS

The trial design of the DINO trial has been reported previously.[8] The DINO trial recruited 657 infants in a blinded, randomized controlled trial conducted at 5 Australian perinatal centers; the primary objective was to determine the effect of meeting the estimated DHA requirement of preterm infants on neurodevelopment. The sample size of 288 children per group was selected to detect a 4-point difference in Mental Development Index scores between the groups at 18 months' corrected age with >85% power ($\alpha = .05$). Long-term follow-up to 18 months and data collection covering a broad range of neonatal outcomes have allowed the reporting of secondary outcomes, including important allergic and respiratory parameters.

The local review boards (human research ethics committees) of each center granted ethics approval for the trial. Infants born before 33 weeks' gestation were eligible, and families were approached within 5 days of the infant commencing any enteral feedings. Infants were excluded if they had major congenital or chromosomal abnormalities, were from a multiple birth in which not all live-born infants were eligible, or were enrolled in other trials of fatty acid supplementation. Lactating mothers in whom tuna oil was contraindicated were also excluded.

After written informed consent was obtained, mother-infant pairs were randomly assigned to receive either a high-DHA or standard-DHA diet through a computerized telephone randomization service. Prerandomization stratification was according to center, birth weight (<1250 or ≥1250 g), and infant gender. Multiple births were considered a single randomization unit, and randomization of twins or triplets was according to the gender and birth weight of the first-born infant. Baseline characteristics were collected, including maternal age, infant race as identified by parents, parental education, birth order, parity, gestational age at birth, birth weight, and pregnancy and birth complications.

Lactating women whose infants were randomly assigned to the high-DHA group consumed six 500-mg DHA-rich tuna oil capsules per day to achieve a breast milk DHA concentration that was ~1% of total fatty acids without altering the naturally occurring concentration of arachidonic acid (AA) in breast milk.[9] If supplementary formula was required, infants were given a high-DHA preterm formula (~1% DHA and 0.6% AA). Mothers with infants allocated to the standard-DHA group consumed six 500-mg placebo soy oil capsules. If supplementary formula was required in this group, a standard preterm infant formula was used (~0.35% DHA and 0.6% AA). The intervention in both groups continued until infants reached their expected date of delivery.

Data collected included survival to discharge, duration of respiratory support via an endotracheal tube, requirement for postnatal steroid therapy, duration of nasal continuous positive airway pressure treatment, days requiring supplemental oxygen, oxygen

TABLE 1 Respiratory Data

All Infants	High-/Standard-DHA Diet, *n*	High-DHA Diet, *n* (%) or Mean (IQR)	Standard-DHA Diet, *n* (%) or Mean (IQR)	Unadjusted RR (95% CI)	Unadjusted *P*	Adjusted RR (95% CI)[a]	Adjusted *P*
Death before discharge[b]	322/335	9 (2.8)	6 (1.8)	1.56 (0.56–4.34)	.39	1.66 (0.63–4.41)	.31
Days on endotracheal support	321/334	6.1 (0–5)	6.8 (0–5)	0.90 (0.63–1.29)	.58	0.88 (0.65–1.18)	.39
Days on CPAP	321/334	9.4 (1–12)	9.1 (0–10)	1.02 (0.79–1.33)	.85	1.04 (0.83–1.30)	.72
Days requiring oxygen (up to EDD)	321/334	21.6 (1–29)	25.5 (1–51)	0.85 (0.67–1.08)	.19	0.82 (0.64–1.06)	.13
Oxygen at 36 weeks[b]	319/334	60 (18.8)	84 (25.1)	0.75 (0.54–1.03)	.07	0.77 (0.59–1.02)	.07
Birth weight <1250 g[c]	145/149	50 (34.5)	70 (47.0)	0.73 (0.55–0.99)	.04	0.75 (0.57–0.98)	.04
Birth weight ≥1250 g[c]	174/185	10 (5.7)	14 (7.6)	0.76 (0.34–1.70)	.50	0.81 (0.37–1.80)	.61
Male infants[d]	171/182	32 (18.7)	51 (28.0)	0.67 (0.45–1.00)	.05	0.67 (0.47–0.96)	.03
Female infants[d]	148/152	28 (18.9)	33 (21.7)	0.87 (0.53–1.42)	.58	0.94 (0.64–1.39)	.76
Infant discharged from hospital with oxygen	318/334	29 (9.1)	37 (11.1)	0.82 (0.51–1.33)	.43	0.87 (0.55–1.36)	.54
Days in NICU[b]	321/334	21.7 (3–31)	21.3 (4–33)	1.02 (0.82–1.27)	.88	1.03 (0.88–1.20)	.75
Days in hospital care[b]	319/332	64.8 (41–80)	64.4 (41–80)	1.01 (0.92–1.10)	.87	1.00 (0.95–1.06)	.88
Any readmission to hospital	300/313	159 (53)	170 (54.3)	0.98 (0.83–1.14)	.76	0.98 (0.85–1.14)	.81
Postnatal steroids[b]	320/332	30 (9.4)	34 (10.2)	0.92 (0.56–1.51)	.73	0.96 (0.61–1.51)	.86

Nonimputed data presented. CPAP indicates continuous positive airway pressure; EDD, expected date of delivery.

[a] Adjusted for gestational age at delivery and for gender (except in gender subgroup analyses).

[b] Previously reported outcome using multiple imputation for missing data.[8] Data presented here are nonimputed; subgroup analysis of oxygen at 36 weeks has not been previously reported.

[c] Adjusted birth weight strata interaction effect: $P = .84$.

[d] Adjusted gender strata interaction effect: $P = .20$.

requirement at a corrected age of 36 weeks' gestation (used as a definition of BPD in all centers), total days in the NICU, discharge from the hospital with oxygen, and total days in hospital care. Structured parental interviews at 12 and 18 months allowed parents to report medical attention for, or the treatment of, hay fever, eczema, asthma, or food allergy. Readmissions to hospital for any reason were also recorded from parental interview.

The outcomes presented here were secondary, and thus there may be insufficient power to detect important differences. All analyses were conducted according to the intention-to-treat principle. The models used to estimate relative risks (RRs) were generalized estimating equations specifying a binomial distribution and log link. Subgroup analyses were performed by testing for an interaction between treatment and subgroup. For this report, nonimputed data were used in all analyses. Outcomes were adjusted for the prespecified potential confounders of infant gender and gestational age at delivery, where appropriate; we provide both unadjusted and adjusted results. The a priori level of significance was $P < .05$; no adjustment was made for multiple testing. Given the number of tests performed and the consequent inflation of type I error, we advise caution in the interpretation of any particular *P* value.

RESULTS

A total of 657 infants were enrolled (high-DHA diet: 322; standard-DHA diet: 335), and 614 infants (93.5%) completed the 18-month follow-up. Although there was a high retention rate in the trial, allergy data were incomplete at 12- and 18-month corrected age, largely because the families who participated in the pilot phase of the trial did not complete the allergy questionnaires[8]; the number of infants for whom data were complete is indicated in the tables. The demographic and clinical characteristics were comparable between the groups.[8] Median duration of treatment was comparable between the high-DHA and standard-DHA groups: 9.4 weeks (interquartile range [IQR]: 7.9–11.4 weeks) versus 9.4 weeks (IQR: 8.0–11.6), respectively. On the basis of capsule returns, maternal compliance was 81.1% in the high-DHA group and 81.7% in the standard-DHA group ($P = .88$).

Overall, there was no difference in risk of BPD between the groups (adjusted RR: 0.77 [95% confidence interval (CI): 0.59–1.02]; $P = .07$) (Table 1). Subgroup analysis based on the randomization strata demonstrated a significant reduction in oxygen requirement at 36 weeks' gestation in infants with birth weight <1250 g (RR: 0.75 [95% CI: 0.57– 0.98]; $P = .04$) and in male infants (RR: 0.67 [95% CI: 0.47–0.96]; $P = .03$), although there were no significant interactions for treatment according to gender ($P = .20$) or birth weight ($P = .84$). No significant differences were found between the groups with regard to discharge from the hospital with oxygen, duration of respiratory support, survival to discharge, duration of admission, need for readmission to hospital, or postnatal steroid use.

There was a reduction in parental report of hay fever noted in the high-DHA group at either 12 or 18 months' corrected age (RR: 0.41 [95% CI: 0.18–0.91]; $P = .03$) (Table 2). Subgroup

TABLE 2 Allergy Data for All Infants

All Infants	High-/Standard-DHA Diet, *n*	High-DHA Diet, *n* (%)	Standard-DHA Diet, *n* (%)	Unadjusted RR (95% CI)	Unadjusted *P*	Adjusted RR (95% CI)[a]	Adjusted *P*
Hay fever					.16		.16
12 mo	232/249	5 (2.2)	13 (5.2)	0.41 (0.15–1.16)	.09	0.41 (0.15–1.16)	.09
18 mo	292/311	7 (2.4)	10 (3.2)	0.75 (0.28–2.00)	.56	0.75 (0.28–2.01)	.57
Either 12 or 18 mo[b]	231/244	8 (3.5)	21 (8.6)	0.40 (0.18–0.91)	.03	0.41 (0.18–0.91)	.03
Asthma					.48		.49
12 mo	232/249	18 (7.8)	25 (10.0)	0.77 (0.42–1.41)	.40	0.77 (0.42–1.40)	.39
18 mo	292/311	41 (14.0)	46 (14.8)	0.95 (0.63–1.43)	.80	0.96 (0.64–1.43)	.83
Either 12 or 18 mo[b]	237/252	47 (19.8)	53 (21.0)	0.94 (0.65–1.36)	.75	0.95 (0.66–1.36)	.78
Eczema					.50		.51
12 mo	232/249	29 (12.5)	40 (16.1)	0.78 (0.49–1.22)	.28	0.78 (0.50–1.22)	.28
18 mo	292/311	48 (16.4)	51 (16.4)	1.00 (0.67–1.49)	.99	1.01 (0.68–1.50)	.97
Either 12 or 18 mo[b]	236/248	61 (25.8)	67 (27.0)	0.96 (0.69–1.33)	.79	0.96 (0.69–1.33)	.81
Special diet for food allergy					.97		.96
12 mo	232/248	12 (5.2)	13 (5.2)	0.99 (0.42–2.31)	.98	0.98 (0.42–2.27)	.96
18 mo	292/311	12 (4.1)	13 (4.2)	0.98 (0.42–2.31)	.97	0.99 (0.42–2.33)	.98
Either 12 or 18 mo[b]	230/243	20 (8.7)	17 (7.0)	1.24 (0.62–2.50)	.54	1.25 (0.62–2.51)	.53

Nonimputed data presented.
[a] Adjusted for gestational age at delivery and for gender.
[b] Excluding missing data.

TABLE 3 Hay Fever Data According to Subgroup

Subgroup	High-/Standard-DHA Diet, *n*	High-DHA Diet, *n* (%)	Standard-DHA Diet, *n* (%)	Unadjusted RR (95% CI)	Unadjusted *P*	Adjusted RR (95% CI)[a]	Adjusted *P*
Birth weight <1250 g							
12 mo	102/107	2 (2.0)	6 (5.6)	0.35 (0.07–1.80)	.21	0.35 (0.07–1.88)	.22
18 mo	133/141	4 (3.0)	5 (3.5)	0.85 (0.23–3.08)	.80	0.85 (0.23–3.13)	.81
Either 12 or 18 mo[b]	101/106	5 (5.0)	10 (9.4)	0.52 (0.18–1.53)	.24	0.52 (0.18–1.53)	.24
Birth weight ≥1250 g							
12 mo	130/142	3 (2.3)	7 (4.9)	0.47 (0.12–1.77)	.26	0.47 (0.12–1.76)	.26
18 mo	159/170	3 (1.9)	5 (2.9)	0.64 (0.16–2.64)	.54	0.64 (0.16–2.62)	.54
Either 12 or 18 mo[b]	130/138	3 (2.3)	11 (8.0)	0.29 (0.08–1.01)	.05	0.29 (0.08–1.02)	.05
Male infants							
12 mo	126/139	1 (0.8)	10 (7.2)	0.11 (0.01–0.87)	.04	0.11 (0.01–0.85)	.03
18 mo	155/168	2 (1.3)	5 (3.0)	0.43 (0.08–2.41)	.34	0.43 (0.08–2.41)	.34
Either 12 or 18 mo[b]	125/137	2 (1.6)	15 (10.9)	0.15 (0.03–0.64)	.01	0.15 (0.03–0.64)	.01
Female infants							
12 mo	106/110	4 (3.8)	3 (2.7)	1.38 (0.32–6.05)	.67	1.42 (0.33–6.16)	.64
18 mo	137/143	5 (3.6)	5 (3.5)	1.04 (0.31–3.52)	.94	1.05 (0.31–3.56)	.93
Either 12 or 18 mo[b]	106/107	6 (5.7)	6 (5.6)	1.01 (0.34–3.03)	.99	1.04 (0.35–3.13)	.94

Nonimputed data presented. Adjusted birth weight strata interaction effect at 12 or 18 months: $P = .49$; adjusted gender strata interaction effect: $P = .05$ at 12 months, $P = .04$ at 12 or 18 months.
[a] Adjusted for gestational age at delivery and for gender (except in gender subgroup analyses).
[b] Excluding missing data.

analysis of this finding (Table 3) revealed a reduction in hay fever approaching significance in infants with a birth weight of ≥1250 g (RR: 0.29 [95% CI: 0.08–1.02]; $P = .05$) and a significant reduction in male infants at 12 months (RR: 0.11 [95% CI: 0.01–0.85]; $P = .03$) and at either 12 or 18 months (RR: 0.15 [95% CI: 0.03–0.64]; $P = .01$). Interaction analyses of these findings supported the reduced incidence of hay fever in male infants at 12 months ($P = .05$) and at 12 or 18 months ($P = .04$) but not the difference found in the birth weight subgroup ($P = .49$). There were no differences in the risk of asthma, eczema, or requirement for special diet for food allergy overall or in any subgroup.

DISCUSSION

The DINO trial is the largest study of DHA supplementation to preterm infants to report longer-term allergic and respiratory outcomes. Our data suggest that DHA supplementation during the preterm period reduces the incidence of BPD in males and infants with a birth weight of <1250 g. We cannot exclude the possibility, however, that these findings are due to chance because of the absence of significant interactions between treatment and infant gender and between treatment and birth weight strata.

Nevertheless, these findings are potentially important and deserve further investigation, because BPD remains an

important predictor of morbidity in the long-term.[10,11] Few interventions have been shown to significantly reduce the incidence of BPD; caffeine is a notable exception.[12] Animal studies have demonstrated that high DHA exposure increases production of the major surfactant lipid, dipalmitoyl phosphatidylcholine (DPPC), in the fetal and neonatal lung. Chao et al[13] demonstrated that DHA and AA supplementation to preterm baboons led to levels of DPPC in the lung equivalent to term controls. Blanco et al[14] produced a similar result in the preterm mouse lung. Along with its antiinflammatory effect, improved surfactant function is a potential mechanism by which DHA supplementation improves pulmonary outcomes.

A Norwegian randomized controlled trial of DHA supplementation to preterm infants included short-term respiratory parameters as secondary outcomes.[15] This trial of 141 infants with birth weight <1500 g intervened with high-DHA and high-AA milk from 1 week after birth until discharge from hospital, and found no significant difference between groups in need of respiratory support, duration of mechanical ventilation, or duration of oxygen support. A nonrandomized study of 1342 non-breastfed infants given either a formula containing DHA plus AA or a control formula[16] found a higher incidence of bronchiolitis in controls than in the LCPUFA DHA group at 5, 7 and 9 months. In this study, the dose of LCPUFA matched the level consumed by the control infants in the DINO trial, whereas the treated infants in DINO received 3 times the DHA level at ~1% of total fatty acids. Other studies of DHA supplementation for preterm infants using formula, or a mixture of formula and breast milk, reported no difference in respiratory outcomes.[17–20]

We found a reduction in risk of reported hay fever with DHA supplementation, which may support a preventative role for allergy. However, we found no reduction in the reported incidence of asthma, eczema, or food allergy, which is unexpected if DHA is acting on immunoglobulin E–related disease. Low levels of *n*-3 LCPUFAs in breast milk have previously been associated with increased risk of infant atopy,[21–23] whereas several studies have found a reduction in asthma or atopic disease in children in whom fish oil or DHA supplementation occurred during pregnancy or breastfeeding.[24,25] The incidence of parent-reported asthma in our study was also much higher than expected (previously reported as 2%–4% for children up to 1 year of age in Australia).[26]

As mentioned previously, the DINO trial was powered to achieve a result in the primary outcome, a difference in Mental Development Index scores at 18 months' corrected age. Thus, the allergic and respiratory results presented here must be interpreted with caution, as the study was not powered to detect a difference in these parameters. Subgroup analysis demonstrated a significant reduction in BPD in the smaller and male infants, but the interaction analysis of these subgroups was not significant. Given the trend toward a reduction in BPD overall with a high-DHA diet ($P = .07$), and an absolute reduction in BPD observed in girls (28% vs 33%) and heavier infants (10% vs 14%) (Table 1), it is possible that in reality a high-DHA diet is beneficial in reducing BPD in all subgroups, but the study was underpowered to demonstrate this. If boys do in fact benefit more than girls from DHA supplementation, a possible explanation is found in adult data reporting females have a higher rate of endogenous DHA synthesis from the precursor fatty acid α-linoleic acid than males,[27] and boys have a faster growth rate and higher metabolic rate than girls,[28] and may be using more of the dietary DHA for energy.

A weakness of our data is that the allergy outcomes relied on parental recall of medical attention or treatment for a condition, rather than a definitive diagnosis based on a recognized test (eg, skin prick testing, peak flow measurements). It may be that the higher than expected rates of asthma and hay fever reported in our study relate more to poorer respiratory function in the population studied, and that the diagnosis of "hay fever" or "asthma" represents overlap with respiratory tract infections. It is well documented that the diagnosis of asthma in infants is problematic[29] because other conditions produce a self-limiting wheeze or cough. The planned follow-up of DINO children at 7 years may help determine the optimal dose of *n*-3 LCPUFA to ameliorate bronchiolitis and other respiratory conditions, and also aid in distinguishing hay fever from other diagnoses. One strength of the DINO trial was that both mothers and those assessing study outcomes were blinded to treatment allocation, reducing the likelihood of bias.

The findings of this study suggest a response to high-dose DHA in the form of decreased hay fever in male infants, and a possible major benefit in reducing the incidence of BPD. These results add weight to the argument for high-dose DHA supplementation in the most at-risk premature infants, and support previous evidence that DHA may have a role in reducing atopic conditions. Future studies should be powered to achieve a difference in BPD incidence in DHA-supplemented infants. The optimal dose of DHA is uncertain, and although the dose used in the DINO trial should be compared with a higher-dose strategy, any products designed for neonates born at <1250 g should contain at least 1% of the total fats as DHA.

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