A dose response randomised controlled trial of docosahexaenoic acid (DHA) in preterm infants


ARTICLE INFO

Keywords:
Infant
Premature
Docosahexaenoic acid
Long chain polyunsaturated fatty acid
Dose response

ABSTRACT

Thirty one infants born less than 30 weeks' gestational age were randomised to receive either 40 (n = 11), 80 (n = 9) or 120 (n = 11) mg/kg/day of docosahexaenoic acid (DHA) respectively as an emulsion, via the feeding tube, commenced within 4 days of the first enteral feed. Twenty three infants were enrolled in non-randomised reference groups; n = 11 who had no supplementary DHA and n = 12 who had maternal DHA supplementation. All levels of DHA in the emulsion were well tolerated with no effect on number of days of interrupted feeds or days to full enteral feeds. DHA levels in diets were directly related to blood DHA levels but were unrelated to arachidonic acid (AA) levels. All randomised groups and the maternal supplementation reference group prevented the drop in DHA levels at study end that was evident in infants not receiving supplementation. Australian New Zealand Clinical Trials Registry: ACTRN12610000382077.

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1. Introduction

Very preterm infants are at significant risk for longer term developmental disorders and learning disabilities [1]. We have shown in infants born less than 33 weeks' gestation that supplementation with docosahexaenoic acid (DHA, an n-3 long chain polyunsaturated fatty acid, LCPUFA) through breast milk or formula at a dose designed to approximate the in-utero accumulation rate results in a significant decrease at a dose designed to approximate the in-utero accumulation rate results in a significant decrease in the proportion of children with severe mental delay at 18 months' corrected age compared with control (The DINO trial) [2]. However, despite supplementation, the DHA erythrocyte phospholipid level at term equivalent age (6.8% of total fatty acids [3]) did not reach levels seen in infants born at term (~8.0% of total fatty acids [4]). Further, we have previously shown for term infants that raising breast milk DHA above 0.8% total fatty acids results in little additional increase in erythrocyte DHA levels plateauing at ~9.0% total fatty acids [5].

A number of factors may have influenced the lower DHA erythrocyte phospholipid levels found in preterm infants in the DINO trial compared with infants born at term. The biological variability and compliance variation in the mothers may have influenced the amount of DHA in the breast milk; the delay in reaching target infant milk volumes common in this population meant that the full dose of DHA was not given until receiving full enteral feeds; in addition there is the potential for losses due to oxidation of DHA for energy. Limitations in the timing of delivery and dose found with maternal or formula supplementation can be overcome by direct supplementation to the infant. It is possible that a higher dose of DHA is required to achieve erythrocyte phospholipid DHA levels seen in term infants. The aims of this study were therefore to 1) determine the dose of oral DHA

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required in a 28 day period to achieve a DHA status not different to that seen in term infants, and 2) to assess the tolerance of direct supplementation.

2. Patients and methods

2.1. Participants

This was a single centre randomised controlled, dose response trial conducted in Adelaide, South Australia. Parents of infants born less than 33 weeks' gestation at the Women's and Children's Hospital were invited to participate in the study. Eligibility criteria included infants after one and before five days of commencing any enteral feeds, and women providing breast milk for their infant not taking supplements containing DHA or willing to stop taking supplements for the duration study. Multiple births were eligible and were randomised individually. Infants with major congenital or chromosomal abnormalities or infants likely to be transferred to remote locations where weekly blood tests could not be done were excluded from the study. The Women's and Children's Health Network Human Research Ethics Committee approved the trial and written informed consent was obtained from parents.

2.2. Randomisation and blinding

A computer generated randomisation schedule using randomly permuted blocks of 6 was generated by a researcher independent of study conduct. Stratification occurred for sex and gestational age (28 weeks and 28–32 weeks). The parents of eligible infants were approached to enter the trial by the study neonatologist or nominee; follow-up for consent was done by the research nurse. Upon consent, study documentation was sent to the clinical trial pharmacist who held the randomisation schedule and randomised infants to one of the three study emulsions assigning a unique study ID. Participants, care providers, outcome assessors and data analysts were blinded to randomisation group.

2.3. Intervention

Infants were randomised to receive one of three oral supplements containing DHA at ≈ 40 mg/mL, 80 mg/mL or 120 mg/mL. The study emulsions were identical in appearance. The extra calorific content of the study emulsion was negligible; 100 mg of study emulsion delivered 1.0 kJ. Clover Corporation Ltd provided the study product which was an emulsion of fractionated tuna oil emulsified with 1% lecithin. The aqueous emulsion contained 18.8% total fat (containing 70% of total oil as DHA in triglyceride form). The base emulsion delivered around 120 mg/mL of DHA, 15 mg/mL of eicosapentaenoic acid (EPA); 0.22 mg/mL alpha linolenic acid and included a trace amount of arachidonic acid (AA). The fat component of the base emulsion was blended with soy oil to achieve the study concentrations.

The study emulsion was administered at 1.0 mL/kg/day in three divided doses to provide 40, 80 or 120 mg/kg/day of DHA. The emulsion was given immediately preceding a scheduled feed through the infant feeding tube. If enteral feeds were stopped, the study emulsion was not given until feeds re-commenced. The dose was recalculated weekly based on birth weight until birth weight was regained, then using the current weight of the infant. The intervention continued for 28 days.

2.4. Reference groups

Two reference groups of infants born less than 33 weeks' gestation were included: infants who did not receive any supplementary DHA and preterm girls whose mothers were taking high dose fish oil capsules. The results of the DINO trial [2] had influenced clinical practice at the Women's and Children's Hospital so that lactating women with preterm girls were provided with fish oil capsules to raise the breast milk DHA level to that achieved in the DINO trial (2 capsules of Blackmore's Omegabrain containing 500 mg DHA and 100 mg EPA per capsule). It was therefore considered unethical to randomise infants to a 'no supplementary DHA' group. Eligibility criteria for the 'no supplementary DHA' reference group therefore included boys born less than 33 weeks' gestation whose mother was not taking DHA supplements or girls born less than 33 weeks' gestation who would only receive formula.

We knew from the DINO trial the erythrocyte status at term equivalent of infants whose mothers' took high dose fish oil capsules [3] but we did not know how quickly this rose, and to what level, over the first weeks of enteral nutrition. We therefore included the second reference group of girls born less than 33 weeks' gestation who were receiving breast milk from women taking DHA supplements according to the hospital protocol stated above.

2.5. Outcomes

The primary outcome was erythrocyte phospholipid DHA levels and the secondary outcome was tolerance of the emulsion. A 400 μL sample of venous blood was collected to determine erythrocyte phospholipid DHA. The blood samples were taken on study entry and at 7 day intervals for 28 days (a total of 5 blood samples). Women providing breast milk for their infant were also asked to supply one 5 mL sample of breast milk during the time of the study for fatty acid analysis.

2.5.1. Fatty acid analysis

The fatty acid analyses were undertaken using established methods [5]. Whole blood was collected into heparinised tubes and centrifuged to separate erythrocyte and plasma fractions. Erythrocyte lipids were extracted in chloroform:propanol. The chloroform layer was removed and evaporated under nitrogen, then phospholipids separated by thin-layer chromatography. Phospholipids were methylated in 1% H2SO4 in methanol at 70 °C for 3 h. The resulting methyl esters were extracted into n-heptane and dehydrated in anhydrous Na2SO4. Fatty acid methyl esters were separated and quantified using a Hewlett-Packard 5880 gas chromatograph equipped with a 50 m capillary column coated with BPX-70 (0.25 μm film thickness, SGE Pty Ltd., Victoria, Australia). The injector temperature was set at 250 °C and detector (flame ionisation) temperature at 300 °C. The initial oven temperature was 140 °C and was programmed to rise to 220 °C at 5 °C/min. Helium was used as the carrier gas at a velocity of 35 cm/s. Fatty acid methyl esters were identified based on retention time to authentic lipid standards obtained from NuChek Prep Inc. (Elysian, MN).

2.5.2. Tolerance

Tolerance of the enteral emulsion was determined by comparing the time (days) taken to reach full enteral feeds (≥ 150 mLs/kg/day of enteral intake) and the number of days on which one or more prescribed feeds were not given in a 24 h period between groups.

2.6. Statistical analysis

2.6.1. Sample size

A sample size of 8 infants per randomised group provided 80% power (alpha=0.05, two-tailed) to detect an increase in mean erythrocyte phospholipid levels at final follow-up from 6.8% of total fatty acids, SD 1.2% (achieved in the intervention group in the DINO trial [3]) to 9.0%, SD 1.5 (achieved in term infants receiving...
breast milk with 0.8% DHA [5]) between the control study emulsion (40 mg DHA/mL) and the highest dose emulsion (120 mg/mL). We aimed to include 10 infants per randomised group and 10 infants per reference group for a total of 50 infants.

Statistical analyses were performed using SAS version 9.3 (SAS Institute Inc., Cary, NC, USA). Infants were analysed according to the group to which they were assigned. Differences in erythrocyte phospholipid fatty acids levels between the five groups over time (baseline, study day 7, study day 14, study day 21, study day 28) were assessed using linear mixed effects models. Randomised group, time and the interaction between group and time were included as categorical fixed effects in the models, while infant was included as a random effect to allow for correlated measurements over time. A random mother effect to account for the potential clustering of infants within mothers was also explored, but this had minimal impact on the results and led to estimation problems in some instances. Where the interaction between group and time was not statistically significant, the interaction term was removed from the model and the main effect of group quantified. Statistical significance was assessed at the two-sided $p < 0.05$ level throughout.

3. Results

Thirty one infants were enrolled between August and November 2010; $n=11, 9$ and $11$ to receive $40, 80$ and $120$ mg DHA/kg/day respectively. Twenty three infants were enrolled in the

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### Table 1
Baseline clinical characteristics$^a$.

<table>
<thead>
<tr>
<th></th>
<th>Randomised</th>
<th>Reference</th>
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<tbody>
<tr>
<td></td>
<td>DHA 40 mg/kg/day</td>
<td>DHA 80 mg/kg/day</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, weeks $&lt; 28$ weeks gestation</td>
<td>29.7 ± 2.0</td>
<td>29.0 ± (1.7)</td>
</tr>
<tr>
<td>Sex – female</td>
<td>2 (18)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>7 (64)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Caesarean section</td>
<td>6 (55)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Birth weight, mean ± SD, grams</td>
<td>1455 ± 485</td>
<td>1240 ± 329</td>
</tr>
<tr>
<td>Multiple</td>
<td>3 (27)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>Age first enteral feed, median (IQR), days $&lt; 1$</td>
<td>1 (1-3)</td>
<td>2 (1-3)</td>
</tr>
<tr>
<td>Age first enteral feed, median (IQR), days $&lt; 5$</td>
<td>5 (4-6)</td>
<td>6 (5-7)</td>
</tr>
<tr>
<td>Days from first enteral feed to first enteral feeding, median (IQR)</td>
<td>4 (3-5)</td>
<td>4 (3-5)</td>
</tr>
</tbody>
</table>

$^a$ NA–not applicable.

$^a$ Data are presented as $n$ (%) unless otherwise indicated.

### Table 2
Compliance and tolerance of study emulsion$^a$.

<table>
<thead>
<tr>
<th></th>
<th>Randomised</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DHA 40 mg/kg/day</td>
<td>DHA 80 mg/kg/day</td>
</tr>
<tr>
<td>Received all doses</td>
<td>8 (73)</td>
<td>7 (78)</td>
</tr>
<tr>
<td>Received $&gt; 85%$ of doses</td>
<td>9 (82)</td>
<td>8 (89)</td>
</tr>
<tr>
<td>Feeds interrupted, median (IQR), days $&lt; 1$</td>
<td>0 (0-1)</td>
<td>0 (0-2)</td>
</tr>
<tr>
<td>Full enteral feeds reached, median (IQR), days$^b$</td>
<td>10 (6-15)</td>
<td>14 (12-19)</td>
</tr>
</tbody>
</table>

$^a$ Data are presented as $n$ (%) unless otherwise indicated.

$^b$ ≥ 150 mLs/kg/day of enteral intake.

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non-randomised reference groups between June and November 2011; n=11 who had no supplementary DHA and n=12 who had maternal DHA supplementation (Fig. 1). Infants were analysed according to their randomised or reference group; one child was withdrawn at the parents request from the no supplementary DHA reference group. Baseline characteristics were similar between all groups (Table 1). Infants were a mean of 29.5 (SD 1.8) weeks’ gestational age, had their first enteral feed at a median of 1 (IQR 1–2) days of age and commenced the study emulsion at a median of 5 (IQR 4–7) days of age. At study end (28 days after commencing the emulsion) infants were a mean of 34 (SD 1.6) weeks post-menstrual age.

3.1. Emulsion compliance and tolerance

Compliance with the study intervention was high with 74% (n=23) receiving all ordered doses and 87% (n=27) receiving > 85% of ordered doses, with no difference between randomised groups (Table 2). The emulsion was well tolerated with no difference between groups in the number of days in which one

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or more feeds were interrupted and the number of days taken to reach full enteral feeds (Table 2).

3.2. Erythrocyte membrane phospholipid LCPUFA

There were no significant differences in fatty acid levels at baseline (Fig. 2) with DHA measuring ≈ 4.7%, Eicosapentaenoic Acid (EPA) ≈ 0.2% and Arachidonic Acid (AA) ≈ 16.7% of total fatty acids. Fatty acid levels at study end are shown in Table 3. There was a significant group by time interaction effect \((P=0.0001)\) for DHA erythrocyte phospholipid levels. Only the highest dose emulsion (120 mg/kg/d) prevented the fall in DHA levels in the infant erythrocytes at study day 7 (a statistically significant difference when compared with unsupplemented and maternal supplementation groups, \(P=0.004\), Fig. 2). Supplementation with 80 and 40 mg/kg/d inhibited the decline in DHA but this did not reach significance (Fig. 2). All randomised groups and the maternal supplementation group by time interaction effect (Fig. 2) with DHA measuring

Table 3
Fatty acid content of RBC phospholipid at 28 days (% of total fatty acid).a

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<th>Randomised</th>
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<tr>
<td></td>
<td>DHA 40 mg/kg/day (\text{day} n=11)</td>
<td>DHA 80 mg/kg/day (\text{day} n=9)</td>
</tr>
<tr>
<td><strong>Saturates</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>45.59 ± 0.45</td>
<td>45.46 ± 0.49</td>
</tr>
<tr>
<td><strong>Monounsaturates</strong></td>
<td>17.73 ± 0.38</td>
<td>18.24 ± 0.42</td>
</tr>
<tr>
<td><strong>Polyunsaturates</strong></td>
<td>5.94 ± 0.56</td>
<td>7.81 ± 0.62</td>
</tr>
<tr>
<td><strong>Fatty acid</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arachidonic</td>
<td>14.65 ± 0.34</td>
<td>14.10 ± 0.38</td>
</tr>
<tr>
<td>Total n-6</td>
<td>28.92 ± 0.61</td>
<td>27.69 ± 0.67</td>
</tr>
<tr>
<td><strong>p&lt;0.05</strong></td>
<td>0.01</td>
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Values with different superscripts are significantly different: \(P < 0.05\).

a Data presented as mean ± SE of fatty acid as a % of total phospholipid fatty acids.

b No evidence for an effect of treatment groups.

c Statistical comparison not performed.

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b No evidence for an effect of treatment groups.

c Statistical comparison not performed.

3.4. Other clinical outcomes

Weight length and head circumference at study end and on discharge home were similar in all groups (results not shown).

The study was not designed to determine the effect on clinical outcomes; however the incidence of major clinical morbidities was low. Bronchopulmonary dysplasia (BPD, defined as oxygen requirement at 36 weeks postmenstrual age) was not present in infants receiving 40 or 60 mg/kg/d group, 2 infants (18%) with 120 mg/kg/d, 1 (9%) with no supplementary DHA and 4 (33%) with maternal supplementation. Necrotising enterocolitis (NEC, defined according to the Australian and New Zealand Neonatal Network [6]) occurred in one infant in each of the 40 mg/kg/d, no supplementary DHA and maternal supplementation groups with no NEC present in the 80 or 120 mg/kg/d groups.

4. Discussion and conclusions

Preterm infants are well recognised as being at risk of DHA dietary insufficiency. Newborn preterm infants have lower plasma and red cell concentrations of DHA compared with newborn term infants [7], and their often complex feeding regimens and feeding intolerances limit intake of a consistent supply of DHA whether through expressed human milk or infant formula. This study demonstrates that direct supplementation of the infants significantly reduced (40 and 80 mg/kg/d DHA) or prevented (120 mg/kg/d DHA) the drop in DHA levels in the first 7 days that was evident in infants that were unsupplemented or had maternal supplementation.

While supplementing women increases the DHA content of their maternal DHA and maternal supplementation groups with no NEC present in the 80 or 120 mg/kg/d groups.

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even longer in the very preterm infants (median 17 days (IQR 26–98 days) (unpublished data). Infants in this current study reached full enteral feeds at a median age of 10–14 days with no evidence of feed intolerance related to the use of the DHA emulsion and with the majority (87%) receiving the full dose of DHA emulsion from 5 days of age. Further time delays in administering the DHA emulsion associated with gaining parent consent and logistics of study conduct would be reduced if an enteral delivery of DHA formed part of normal clinical practice with the emulsion being given with the first feeds. Thus, direct enteral delivery of DHA through an aqueous emulsion appears to be a well-tolerated and effective way of addressing the DHA requirements of the preterm infant.

Infants born very preterm remain at a distinct disadvantage compared to term born infants despite significant technological, medical and nursing advances that have assured increased survival rates [8]. Because mortality rates have fallen, the focus for perinatal interventions must be the development of strategies to reduce long-term morbidity, especially the prevention of brain injury and abnormal neurodevelopment. We, and others, have confirmed the importance of dietary DHA as a nutritional intervention resulting in improved cognitive and clinical outcomes in very preterm infants [2,9–11] even when the increase in erythrocyte phospholipid DHA level failed to reach that of infants born at term. The current study confirms that the DHA status of very preterm infants can be improved in a dose dependant manner and that enteral DHA supplementation directly to the infant rather than through human milk or formula represents a safe and effective intervention. This study directly addresses factors potentially limiting the beneficial effects of DHA supplementation in preterm infants, including biological variability and compliance variation inherent in maternal supplementation. As such it is an important step towards future randomised controlled studies with improved methodology and potentially greater clinical benefit.

Acknowledgements

We thank the families who generously participated in this study and neonatal research nurses Louise Goodchild and Ros Lontis.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.plefa.2015.04.003.

References


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