Nutritional intervention and neurodevelopmental outcome in newborns at risk of neurodevelopmental impairment: the Dolphin neonatal double-blind randomized controlled trial

MORAG J ANDREW[1*]

JEREMY R PARR[2*]

CHRISTINE MONTAGUE-JOHNSON[1]

KAREN LALER[1]

JANE HOLMES[3]

BONNY BAKER[1]

PETER B SULLIVAN[1]

1 Department of Paediatrics, University of Oxford, Oxford; **2** Institute of Neuroscience, Newcastle University, Newcastle; **3** Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK.

Correspondence to Peter B Sullivan, Department of Paediatrics, University of Oxford, Oxford OX3 9DU, UK. E-mail: peter.sullivan@paediatrics.ox.ac.uk

***These authors share first authorship.**

ABBREVIATIONS

Bayley-III	Bayley Scales of Infant Development, Third Edition
CCS	Cognitive composite score
DHA	Docosahexaenoic acid
LCS	Language composite score
MCS	Motor composite score
UMP	Uridine-5-monophosphate
VABS-II	Vineland Adaptive Behavior Scales, Second Edition

[Abstract]

AIM To investigate whether neonates at risk for neurodevelopmental impairment have improved neurodevelopment after docosahexaenoic acid, choline, and uridine-5-monophosphate supplementation versus controls.

METHOD Recruitment was from UK neonatal units. Eligible for inclusion were infants born at less than 31 weeks' gestation with a weight less than the ninth centile; infants born at less than 31 weeks' gestation with a grade II or higher intraventricular haemorrhage/preterm white matter injury; infants born between 31 and 40 weeks'gestation plus 28 days with a grade II or higher intraventricular haemorrhage/preterm white matter injury, moderate or severe hypoxic ischaemic encephalopathy, or defined neuroimaging abnormalities. Treatment/control supplementation was for 2 years (double-blind, randomized, controlled design). Infants were stratified according to sex, gestation, and brain injury severity. Primary outcome was cognitive composite score (CCS) of the Bayley Scales of Infant Development, Third Edition (Bayley-III) at 24 months). Secondary outcomes were language composite score (LCS) of the Bayley-III, motor composite score (MCS) of the Bayley-III, and Vineland Adaptive Behaviour Scales, Second Edition (VABS-II) score.

RESULTS Sixty-two neonates were recruited, 59 were randomized (34 males, 25 females). Fifty-three started supplementation. Most families found supplementation acceptable. The treatment group CCS-Bayley-III scores were non-significantly higher than controls (mean score difference at 24 months: 9·0; 95% confidence interval –0·2 to 18·2). Language and VABS-II scores, but not motor score, were non-significantly higher in the treatment group.

INTERPRETATION Most families found supplementation feasible. Improved neurodevelopmental outcomes in the treatment group were not statistically significant. A larger multicentre trial exploration is warranted.

WHAT THIS PAPER ADDS

- Dietary supplementation of neonates at risk of neurodevelopmental impairment is feasible.
- No statistically significant neurodevelopmental advantages were identified for the treatment group compared to controls.
- Treatment group cognitive and language advantage are of a clinically meaningful magnitude.

Rapid brain growth and development during the third trimester of pregnancy and the first 2 years of life relies upon adequate macro- and micronutrient intake. Brain integrity and function depends upon specific membrane phospholipids and their fatty acids. Phosphatidylcholine is the most abundant brain phospholipid.[1] The omega-3 fatty acid docosahexaenoic acid (DHA), choline (an amine), and uridine-5-monophosphate (UMP; a pyrimidine) combine to form phosphatidylcholine.[2] DHA, choline, and uridine supplementation synergistically increases rodent brain phospholipids, synaptic components, functional brain connectivity, and cognitive performance.[3]

Micronutrients, including zinc, the B vitamins, and iodine, also have important central nervous system roles. Zinc is important for neurogenesis, neuronal migration and synaptogenesis, and the metabolism of thyroid hormones.[4] The B vitamins also support synaptogenesis and synaptic function. Iodine is necessary for thyroid hormone production; the neurodevelopmental consequences of hyopthyroidism are well recognized. After brain injury, plasticity may contribute to functional recovery, supported by the synaptic elements, extracellular matrix, and guidance molecules of the subplate.[5]

Perinatal factors including extreme prematurity (gestational age <28wk), intrauterine growth restriction (birthweight <10th centile for gestational age), and term hypoxic ischaemic encephalopathy are associated with suboptimal neurodevelopmental outcomes and specific diagnoses, including cerebral palsy, epilepsy, visual impairment, intellectual disability, specific learning and attention difficulties, and neuropsychiatric conditions.[6–9] A systematic review of early intervention on preterm cognitive and motor outcomes identified postintervention cognitive benefit in infancy and at preschool age, and postintervention motor benefit in infancy, although the effect size on motor benefit was small. Heterogeneity between studies prevents adequate comparisons between intervention methods, limiting the ability to draw conclusions about which intervention is most effective.[10] A subsequent review specifically focusing on motor development interventions delivered by parents and continuing after discharge also favoured intervention, with the strongest effects below 6 months corrected age; again, heterogeneity between studies limited interpretation.[11] Therapeutic hypothermia is the only intervention shown to improve mortality and neurodevelopmental outcomes after mild-to-moderate hypoxic ischaemic encephalopathy.[12] The Dolphin neonatal trial and parallel Dolphin infant trial[13]are the first to provide infants with risk factors for neurological impairment with the combination DHA, choline, and UMP at maximum permissible levels during brain development. We investigated whether treatment

group neurodevelopmental outcomes were improved versus controls, and if supplementation was feasible for parents and infants.

METHOD

The Dolphin neonatal trial was a randomized, double-blind, placebo-controlled trial of DHA, choline, and UMP, or control, supplementation in neonates with neurodevelopmental impairment risk factors. The full methodology is available in the published open-access trial protocol.[14]

Participants

All infants had neurological impairment risk factors. Eligible infants were born at less than 31 weeks' gestation with a birthweight less than the ninth centile; infants born at less than 31 weeks' gestation with a grade II or higher intraventricular haemorrhage/preterm white matter injury; infants born between 31 and 40weeks' gestation and 28 days with a grade II or higher intraventricular haemorrhage/preterm white matter injury, moderate or severe hypoxic ischaemic encephalopathy, or defined neuroimaging abnormalities consistent with perinatal brain injury (see Table I).[14] Variation in brain injury inclusion criteria for infants born at term and preterm reflects the predominant patterns of cerebral injury occurring at different stages of brain development through gestation.[15]

Trial exclusion criteria were infants with major congenital malformation; underlying progressive neurological, genetic, or metabolic condition; severe hearing loss; gastrointestinal malabsorption; and cow's milk or egg allergy.

Recruitment was from three UK neonatal units: the John Radcliffe Hospital (Oxford), the Royal Berkshire Hospital (Reading), and Wexham Park Hospital (Slough). Eligible infants were identified by clinical teams with research team support (MJA and CM-J). Interested parents received a parent information sheet, followed by research team contact to answer queries approximately 48 hours later. Written informed consent was obtained from the parents or guardians of participating infants. Ethical approval for the study was granted by the Oxfordshire Research Ethics committee B.

After consent, participants were randomized by the University of Oxford's Centre for Statistics in Medicine, using the minimization factors of sex, gestation (<31 weeks or 31–40weeks and 28 days), and brain injury severity (normal-to-mild, moderate, or severe) identified by brain magnetic resonance imaging or cranial ultrasound scans,[14] conducted as part of routine clinical care. The principal investigators and researchers conducting neurodevelopmental assessments were blind to group allocation. The trial dietician was not blinded, to ensure prompt identification of any side effects of supplementation. The trial administrator was blinded until the time of dietary intake data entry, when group allocation became clear. Unblinded team members did not discuss the infants' treatment group with other team members or parents and had no role in data analysis.

Randomized infants received treatment or control supplementation daily for 2 years from trial entry. The study supplements were produced and quality control checked by Nutricia Advanced Medical Nutrition (Utrecht, the Netherlands). The treatment supplement contained DHA (1% of total daily estimated fatty-acid intake), eicosapentaenoic acid, arachidonic acid, choline, UMP, cytidine monophosphate, vitamin B12, zinc, and iodine (Table II). Treatment and control supplements were produced on a background of infant formula; therefore, the control supplement also contained vitamins, minerals, and trace elements, and a small amount of choline. Infants received a 2g/kg/day supplement (maximum 24g/day), for a total of 2 years from trial entry, or while compliant with the study protocol. Supplementation began once infants were on full milk feeds. The supplement was supplied as a combination of 2g, 3g, and 12g foil sachets to protect it from sunlight. The supplement was mixed with usual formula or expressed breast milk, or wet foods on weaning, and given orally, or through the child's feeding tube. The trial dietician telephoned families every 2 weeks initially, then as required, and visited every 3 months to assess adherence to and tolerance of the supplement, dietary macro- and micro-nutritional intake, growth, and advise on optimal macronutrient intake for both groups.

Study assessment psychometric properties and administration are described in the trial protocol.[14] The Bayley Scales of Infant Development, Third Edition (Bayley-III) were administered to infants at baseline, and at 12 months and 24 months in the child's home by one of two trained assessors (MJA and CMJ). The Vineland Adaptive Behavior Scales, Second Edition (VABS-II) were administered to parents after Bayley-III assessment (MJA or CMJ). Neurophysiological and functional behavioural visual measures were performed at baseline, and at 6 months, 12 months, and 24 months. Occipito-frontal circumference was

measured using a Lasso-o tape measure at baseline then every 3 months. A mean from three occipito-frontal circumference measurements was calculated unless an infant was not compliant, in which case one confidently obtained measurement was accepted. Height, weight, and triceps skinfold thickness were measured every 3 months.[14]

Dietary assessment occurred every 3 months, at home. Dietary history using food recall over the previous week was recorded, and used to advise families on optimal macro- and micronutrient intake. Dietary data were analysed using Dietplan 6 (Forestfield Software, Horsham, UK).

Participant whole-blood fatty-acid samples were collected by heel prick onto a Guthrie card at baseline and at 24 months. Maternal whole-blood fatty-acid levels were collected by finger prick at baseline. Blood fatty-acid levels were analysed using the method of Marangoni et al.[16]

The primary outcome was cognitive composite score (CCS) of Bayley-III after supplementation for 24 months. The Bayley-III is a standardized neurodevelopmental measure, with cognitive, language, and motor domains, for infants aged between 1 month and 42 months.[17] The Bayley-III composite score has a range of 40 to 160 (mean 100, SD 15) and is a normalized transformation of standardized scaled scores, composite scores, and centile ranks provided in the Bayley-III manual.

Secondary neurodevelopmental outcomes included CCS-Bayley-III at 12 months, language composite score (LCS) of the Bayley-III and motor composite score (MCS) of the Bayley-III at 12 months and 24 months, and VABS-II standard adaptive score at 12 months and 24 months. The VABS-II Parent/Caregiver Rating Form is a standardized parent questionnaire regarding adaptive behaviour from birth to the age of 90 years.[18] The measure provides a standard adaptive composite score standardized and normed for age (mean 100, SD 15), derived from standardized domain scores: communication, socialization, and daily living skills.

Statistical analysis

Statistical analyses were conducted according to the trial statistical analysis plan and performed using R version 3.2.1.[19]

Sample size was calculated for 80% power to detect a 12·5-point difference in CCS-Bayley-III, at the 5% level for a two-tailed test. In total, a sample size of 48 participants were required, assuming a SD of 15. Assuming a 20% loss to follow-up the recruitment target was increased to a total of 60 infants.

An intention-to-treat analysis included all infants as randomized who had Bayley-III data at baseline and at least one other time point (n=45). Change in CCS-Bayley-III from baseline was analysed at 12 months and 24 months of supplementation using mixed-effects linear regression to account for repeated measures over time, with model covariates of baseline CCS-Bayley-III, time point (12 months and 24 months postsupplementation), treatment group, an interaction between time point and treatment group, and minimization factors of brain injury severity (normal-to-mild/moderate/severe), gestation (<31wk/31–42wk), and sex (male/female). No adjustment of p values for multiple end points was made.

Analyses of secondary outcomes including LCS-Bayley-III, MCS-Bayley-III, and VABS-II score followed the procedure for the primary outcome analysis. Anthropometric measurements were converted to z-scores using the World Health Organization's Child Growth Standards R 'igrowup' package for infants born at term,[20,21] and the Microsoft Excel add-in LMSgrowth with UK preterm reference data for infants born preterm.[22] Mixed-effects regression models were fitted to the data, and the fitted difference in means between treatment and control groups at 12 months and 24 months presented alongside 95% confidence intervals (CIs) and the associated two-sided p value for the overall effect of treatment.

An independent data-monitoring committee, consisting of two consultant paediatric neurologists, an independent statistician, and the trial statistician, periodically oversaw trial progress and reviewed the results of an interim analysis at the midpoint of the trial.

The trial was registered with the ISRCTN Registry (ISRCTN39264076).

RESULTS

Recruitment was carried out between September 1st, 2009 and January 9th, 2013. In total, 258 families were identified as eligible, of which 126 families were approached. We received consent for 62 infants to participate: 59 were randomized (34 males, 25 females); one infant

died before randomization; and two withdrew after consent was given but before randomization. Fifty-three infants started supplementation. In total, 132 families of eligible infants could not be approached owing to factors including transfer to a non-participating district general hospital, death before approach, language barriers, and issues of parental capacity. The flow of participants is summarized in Figure 1.

The baseline characteristics of the treatment and control group infants are shown in Table III.

Infants who discontinued supplementation before 24 months and did not participate in subsequent follow-up assessments were considered to be withdrawals. There were a total of 16 withdrawals over the 24 months (five from the treatment group and 11 from the control group). Eleven infants were withdrawn before 6 months. By 12 months, a total of 14 infants had withdrawn (four from the treatment group and 10 from the control group). Between 13 months and 24 months two further infants withdrew. One further infant discontinued supplementation but continued with study assessments (data included in analysis). Withdrawal reasons were family circumstance, other appointments, cow's milk protein intolerance, moving country/region, and the effect of comorbidity (predominantly epilepsy) on family life.

Trial supplement intake varied between participants. Of the randomized infants, 39 out of 59 (66%) completed full dose supplementation for 24 months; one infant completed approximately 50% dose for 24 months. Five infants completed full dose for at least 12 months. Five infants completed between 8 weeks and 12 months of supplementation. Three infants were supplemented for less than 8 weeks before withdrawing. Six infants withdrew before supplementation started (one from the treatment group and five from the control group).

After a protocol violation, one infant was randomized to the control group but received treatment supplement for 2 years; this child's data are analysed with the control group.

The long-chain polyunsaturated fatty acid levels in infants in the baseline treatment and control groups were similar. Mean 24-month blood DHA level was higher in the treatment group versus controls (relative percentage 0·009 [SD 0·006] and 0·004 [SD 0·002] respectively; p=0·002), as were mean 24-month eicosapentaenoic acid level (relative percentage 0·031 [SD 0·018] and 0·013 [0·006] respectively; p<0·001).

CCS-Bayley-III data were available for 55 out of 59 (93%) participants. Participants included in the intention-to-treat analysis had CCS-Bayley-III data at baseline and at 12 months or 24 months (*n*=45; 24 from the treatment group and 21 from the control group). Mean baseline CCS-Bayley-III scores were similar for treatment group and control infants. At 12 months, mean CCS-Bayley-III scores were non-significantly higher for the treatment group than controls 85.2 (SD 18·1) versus 82·4 (SD 20 ·1) (mean modelled difference 4·4 points, 95% CI –4·7 to 13·4). At 24 months, CCS-Bayley-III scores were also non-significantly higher in the treatment group than in controls (87·7 [SD 20·4] vs 81·6 [SD 18·5]; mean modelled difference 9·0 points, 95% CI –0·2 to 18·2 [*p*=0·13]; Table IV). There was no difference in treatment effect by visit (no interaction; χ^2[1]=1·64 [*p*=0 ·20]) and no overall effect of treatment (χ^2[1]=2 ·28 [*p*=0·13]).

Post hoc analysis of the primary outcome data after removal of the minimization factors from the model did not change the overall findings (Table IV; mean modelled difference 3·7 points [95% CI –6·4 to 13·8] at 12 months and 8·3 points [95% CI –1·9 to 18·6] at 24 months). There was no difference in treatment effect by visit (χ^2[1]=1·62 [*p*=0·2]) and no overall effect of treatment (χ^2[1]=14·4 [*p*=0·23]). A sensitivity analysis was performed to look at any differential effect of supplementation in infants with mild or moderate brain injury versus those with severe brain injury, in relation to primary outcome. When infants with severe brain injury were removed from analysis, the mean treatment group CCS-Bayley-III remained higher than controls at 24 months (9·25 [SD 19·9] vs 88·8 [SD 14·0]; mean modelled difference 8·7 [95% CI –2·7 to 20·0]).

To investigate whether the observed pattern of cognitive advantage in the treatment group could be the result of higher breastfeeding levels, the primary analysis was repeated, including a main effect for breastfeeding and an interaction term for breastfeeding and treatment, as well as adjusting for baseline Bayley-III cognitive score, treatment group, visit, treatment, and visit interaction, and the minimization factors of brain injury severity, sex, and gestational age at birth. Thirty-three infants (16 in the treatment group and 17 in the control group) were breastfed at baseline (partially or exclusively breastfed for any duration). There was no difference in treatment effect on CCS-Bayley-III at 24 months between those who were breastfed and those who were not (*p*=0·56).

Mean LCS-Bayley-III scores were non-significantly higher for treatment compared with control infants at 12 months (77.6 [SD 14·6] vs 74·6 [SD 12·0]; modelled difference in

means 2·7 [95% CI –6·7 to 12·1] and 24 months (91·5 [SD 20·1] vs 83·2 [SD 19·6]; modelled difference in means 8·6 [95% CI –1·1 to 18 ·2]). There was no overall treatment effect (χ^2[1]=1·57 [*p*=0·21]) or treatment effect by visit (χ^2[1]=1·78 [*p*=0·18]; Table IV).

As with the primary outcome measure of CCS-Bayley-III, post hoc analysis of the data without minimization factors produced similar results (Table IV; mean modelled difference 3·2 points [95% CI –6·2 to 12·7] at 12 months and 9·0 points [95% CI –0·6 to 18·6] at 24 months). There was no difference in treatment effect by visit (χ^2[1]=1·75 [*p*=0·19]) and no overall effect of treatment (χ^2[1]=1·87 [*p*=0·17]).

Mean MCS-Bayley-III scores were similar at 12 months and 24 months for treatment and control groups (Table IV).

Bayley-III composite domain standard scores by group and visit, plus unadjusted and adjusted analyses, are shown in Table IV.

Regarding parent-reported data, mean VABS-II domain standard scores were non-significantly higher in the treatment group compared with control group infants at 12 months (86·9 [SD 16·0] vs 84·6 [SD 13·6]; mean modelled difference 1·6. [95% CI –5·7 to 8·8]) and 24 months (92·3 [SD 10·8] vs 87·4 [SD 15·1]; mean modelled difference 4·4 [95% CI –3·1 to 12·0]). There was no overall treatment effect (χ^2[1]=0·69 [p=0·4]) or treatment effect by visit (χ^2[1]=0·71 [*p*=0·4]).

Reanalysis of VABS-II domain standard scores without minimization factors included in the model did not significantly alter the results. At 12 months the mean modelled treatment group score was 1·9 (95% CI –5·9 to 9·8) and at 24 months it was 4·6 (–3·5 to 12·7). There was no effect of treatment by visit (χ^2[1]=0·60 [*p*=0·44]) and no overall effect of treatment (χ^2[1]=0·73 [p=0·39]).

Forest plots of Bayley-III primary and secondary outcome scores and VABS-II scores are shown in Figure 2.

Owing to differential drop out of infants with normal-to-mild brain injury from the control group, the treatment group as a whole had a less severe brain injury profile. By 24 months, there were 10 treatment group and three control group infants with normal-to-mild brain injury severity. There were similar numbers of infants with moderate brain injury (six

infants in the treatment group and nine in the control group) and severe brain injury (eight infants in the treatment group and seven in the control group) at 24 months.

A comparison of occipito-frontal circumference between baseline and 24 months showed no significant differences between the treatment and control groups (modelled difference in mean z-score at 24 months 0·4; 95% CI –0·1 to 0·9 [p=0·19]). There was no between-group differences in height, weight, or triceps skinfold thickness at 12 months or 24 months (data not shown).

Overall, secondary outcome vision measures showed no differences between the treatment and control groups. Trial vision data will be reported separately (data not shown).

There were no differences in the number of days ventilated nor in the number of infants who developed severe infection or necrotizing enterocolitis between treatment and control groups.

DISCUSSION

This novel trial is the first to provide all three phosphatide precursors (DHA, choline, and UMP) at maximum permissible levels to neonates at risk of neurodisability. Supplementation aimed to achieve DHA levels approaching the maximum naturally occurring levels in human breast milk. Previous studies have used relatively small doses of DHA for relatively short periods of time.[23] Supplementation started around term, and continued throughout the first 2 years of life – the period of maximal brain growth. Supplementation was acceptable to most parents and was continued for 2 years by the majority.

No statistically significant differences in cognitive or language performance were identified between treatment groups. Despite this, mean treatment group cognitive and language scores were higher than in the control group. Similarly, parent-reported VABS-II domain standard scores were higher in the treatment group. The mean difference in CCS-Bayley-III and LCS-Bayley-III are of a clinically significant magnitude – the between-group difference in primary outcome equates to a treatment effect size of 0·31. These effects do not result from macronutrient intake (optimized for both groups); energy and protein intakes were similar for the treatment and control groups and there were no treatment group differences in height or weight. Had the treatment group had better overall nutrition then this

should have been visible as improved treatment group growth parameters over the extended time frame of the study. Furthermore, there was no observed increase in head circumference (brain size) among infants in the treatment group; head circumference was similar at baseline and 24 months for both groups.

The results of previous studies examining the neurocognitive effects of DHA supplementation during early infancy differ, possibly owing to variation in design. The DINO randomized control trial of high-dose DHA (1% of total daily fatty acids) supplementation in preterm neonates reported improvements in Bayley Scales of Infant Development, Second Edition Mental Development Index score in supplemented females (mean difference of 4·5 points in adjusted analyses) but not males. There were fewer infants with impaired neurocognitive development in the treatment group compared with controls.[24] Lucas et al. report cognitive advantage in infants born preterm at less than 30 weeks' gestation fed a 'high-nutrient' or 'standard-nutrient' formula milk (median supplementation 4 weeks), with a mean treatment advantage of 8·6 points in the Bayley Scales of Infant Development, Second Edition Mental Development Index at 18 months corrected gestational age.[25] Bayley Scales of Infant Development, Second Edition performance was best in those infants with the highest intake of the 'high-nutrient' formula, in infants who were small for gestational age and in male infants.[26] The 'high-nutrient' formula supplemented macro- and micronutrient intake; it is not possible to ascertain whether macro- or micronutrient supplementation singly or in combination resulted in the observed treatment group cognitive advantage. As macronutrient intake in the Dolphin control and treatment groups were comparable, any treatment advantage is not the result of improved overall nutritional status. The observed treatment advantage in the Dolphin neonatal trial is comparable with that reported by Lucas et al.[26] Long-term follow-up of the cohort of Lucas et al. confirms lasting neurodevelopmental benefit in the treatment group at school age.[27-30]

This novel multi-site trial recruited infants from UK National Health Service neonatal units. Retention was high: of the 53 families who started supplementation, 45 (85%) continued to at least 12 months. Infants were compliant with supplementation. Formal compliance measures were not included in this study as it was decided that study demands upon participating families were already high; however, supplementation led to higher relative blood DHA and eicosapentaenoic acid levels in the treatment group versus controls, suggesting treatment fidelity was acceptable. Formal compliance measures, such as the

recording of number of returned sachets, would be beneficial in any larger study. Outcome measurement was completed by one of two assessors.

There are a number of challenges for the interpretation of the observed differences in mean CCS-Bayley-III and LCS-Bayley-III. There was heterogeneity regarding gestational age at birth and aetiology, anatomical location, and severity of brain injury. Minimization allowed reasonable comparison between intervention groups; however, larger numbers of infants and narrower inclusion criteria would further reduce heterogeneity between groups. Owing to the number of infants in this study, we could not control for all potential confounding factors, such as maternal education level, which, by chance, was higher in the treatment group than in the control group; this may have contributed to the treatment group neurodevelopmental advantage. Similarly, several other clinical risk factors may have contributed to group neurodevelopmental outcomes; however, there were no differences in the number of days ventilated nor in number of infants who developed severe infection or necrotizing enterocolitis between treatment and control groups. There were no observed treatment group differences in primary outcome measure between infants who were breastfed and those who were not, although statistical tests for interaction have a low power to detect differences.[31] The effect of breastfeeding on treatment group outcome requires further exploration in any future trial. During the supplementation period, there were disproportionately more withdrawals of control infants within the mildest brain injury category, producing a more severe neuroanatomical brain injury profile across controls. Despite statistical modelling accounting for brain injury severity, the observed cognitive and language treatment group advantage may reflect the milder overall brain injury profile of the treatment group.

Despite these challenges to interpretation, if the observed improvement in CCS-Bayley-III for treated infants is replicated in a larger trial, this will have important implications for the treatment of infants at risk of neurological impairment, and for our understanding of the role of brain phosphatide precursors in neurodevelopment. The Dolphin cohort follow-up trials assessing the effects of combination nutritional supplementation in the preschool and school years will provide important long-term follow-up data.

ACKNOWLEDGEMENTS

PBS has received lecture fees from Nutricia Ltd and is a member of the Advanced Medical Nutrition Scientific Advisory Board. MJA has received sponsorship from Nutricia to attend scientific meetings. Other authors have no potential conflict of interest to declare. With thanks to the participating parents and infants; Nicola Alder, Andrew Wilkinson, Bridget Lambert, Angharad Vernon-Roberts, Dr Gerardine Quaghebeur, Dr Tony McShane, Dr Sandeep Jayawant, and Louise Linsell; site Principal Investigators; and consultant neonatologists and paediatricians, nurses, research nurses, and other staff at all sites. We also thank Professors Janette Atkinson and Oliver Braddick for their significant contributions both as collaborators on the visual aspects of the trial and for their role as consultants to the trial more generally. The Dolphin neonates' trial was supported by grants from The Castang Foundation, the Oxford Biomedical Research Centre, and the Thames Valley Clinical Research Network. The supplements were developed and supplied at no cost by Nutricia Ltd, based in The Netherlands. Nutricia Ltd had no role in the development of the protocol, the planning or running of the trial, or the analysis of data. Nutricia provided information about the supplement but did not otherwise contribute to the content of the manuscript. No fees were received from Nutricia Ltd by any of the authors for involvement in this study nor for drafting the manuscript.

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Table I: Neuroimaging severity grading[12]

	Normal/mild	Moderate	Severe
Preterm injury cUSS	• Normal • Grade I/II IVH • VIn <13mm at TEA or • VIn <97th centile for CGA	• Grade III IVH • Non-cystic PVL • VIn 13–15mm TEA or • VIn >97th centile but <4mm above 97th centile for CGA	• Grade IV IVH • PVHI • Cystic PVL • Subcortical leucomalacia • VIn at TEA >15mm or • VIn >4mm above 97th centile for CGA • BG lesions • Focal infarction
Term hypoxic ischaemic encephalopathy MRI (cUSS where MRI unavailable)	• Focal subtle abnormalities of BG with normal appearance of the PLIC • Periventricular white matter changes difficult to differentiate from normal appearances and therefore not classified as abnormal • Changes confined to cerebral cortex and subcortical	• Multi-focal lesions in BG with equivocal or abnormal signal intensity within PLIC • Small focal lesions of without loss of GM/WM differentiation	• Widespread abnormalities involving all BGT structures and PLIC • Larger areas of abnormality with loss of GM/WM differentiation, consistent with infarction • Central grey matter hyperechogenicity ± more extensive cortical and

	WM		subcortical hyperechogenicity
Term infarction MRI (cUSS where MRI unavailable)		• Focal, non-territorial infarct	• Territorial infarct

cUSS, cranial ultrasound scan; IVH, intraventricular haemorrhage; VIn, ventricular index; TEA, term-equivalent age; CGA, corrected gestational age; PVL, periventricular lecuomalacia; PVHI, periventricular haemorrhage infarction; BG, basal ganglia; MRI, magnetic resonance imaging; PLIC, posterior limb of the internal capsule; WM, white matter; GM, grey matter; BGT, basal ganglia–thalamus.

Table II: Active ingredients in the treatment and control supplement (in 2g powder)

Component	Amount in 2g powder	
	Treatment	Control
Docosahexaenoic acid (mg)	37.8	0.4
Eicosapentaenoic acid (mg)	7.8	0.08
Arachidonic acid (mg)	4.4	0.02
Uridine monophosphate (mg)	1.8	0
Cytidine monophosphate (mg)	1.8	0
Choline (mg)	10.5	1.38
Vitamin B12 (μg)	0.12	0.02
Zinc (mg)	0.76	0.06
Iodine (μg)	15	0.6

Table III: Baseline characteristics of randomized participants

	Treatment	Control
Numbers assigned treatment	29	30
Sex (male/female)	17/12	17/13
Gestation (<31 weeks/31–40weeks and 28 days)	14/15	14/16
Mean (SD) gestational age at birth (wk)	32.9 (6.06)	33.7 (6.64)
Neurological damage (mild/moderate/severe)	12/7/10	11/9/10
Median (IQR) birthweight (g)	2095 (950–3040)	2278 (948–3718)
Mean (SD) head circumference (cm)	35.6 (2.52)	36.3 (3.09)
Maternal educational qualification (qualification at 16y/at 18y/tertiary-level education)	3/4/17	1/8/9
Mean (SD) corrected age at baseline (wk)	2.25 (2.67)	3.32 (4.37)
Mean (SD) CCS-Bayley-III	74.29 (13.86)	77.78 (12.81)
Mean (SD) LCS-Bayley-III	66.39 (9.50)	68.48 (10.79)
Mean (SD) MCS-Bayley-III	82.00 (9.20)	82.93 (11.46)

Data are *n* unless otherwise indicated. IQR, interquartile range; CCS, cognitive composite score; Bayley-III, Bayley Scales of Infant Development, Third Edition; LCS, language composite score; MCS, motor composite score.

Table IV: Comparison of Bayley Scales of Infant Development, Third Edition (Bayley-III), composite domain standard scores by group and visit

Outcome measure	Trial visit (mo)	Number included[a]		Mean score (SD)		Difference in mean scores (95% CI)	
		Treatment	Control	Treatment	Control	Unadjusted[b]	Adjusted[c]
CCS-Bayley-III	12	24	21	85.2 (18.1)	82.4 (20.1)	3.7 (–6.4 to 13.8)	4.4 (–4.7 to 13.4)
	24	24	19	87.7 (20.4)	81.6 (18.5)	8.3 (–1.9 to 18.6)	9.0 (–0.2 to 18.2)
LCS-Bayley-III	12	24	21	77.6 (14.6)	74.6 (12.0)	3.2 (–6.2 to 12.7)	2.7 (–6.7 to 12.1)
	24	24	19	91.5 (20.1)	83.2 (19.6)	9.0 (–0.6 to 18.6)	8.6 (–1.1 to 18.2)
MCS-Bayley-III	12	24	20	79.6 (18.3)	80.0 (21.4)	0.1 (–11.6 to 11.8)	–0.2 (–10.6 to 10.3)
	24	23	19	77.8 (21.8)	78.8 (21.6)	–1.5 (–13.4 to 10.4)	–1.2 (–11.9 to 9.5)

[a]All participants included in analysis have a measurement for Bayley composite domain standard score at baseline and at least one other time point. [b]Unadjusted analyses are adjusted for visit and the interaction between treatment and visit. [c]Adjusted for baseline measurement, treatment, visit, interaction between treatment and visit, and the minimization factors of neurological severity, sex, and length of gestation. CI, confidence interval; CCS, cognitive composite score; LCS, language composite score; MCS, motor composite score.

Figure 1: Trial CONSORT flowchart.

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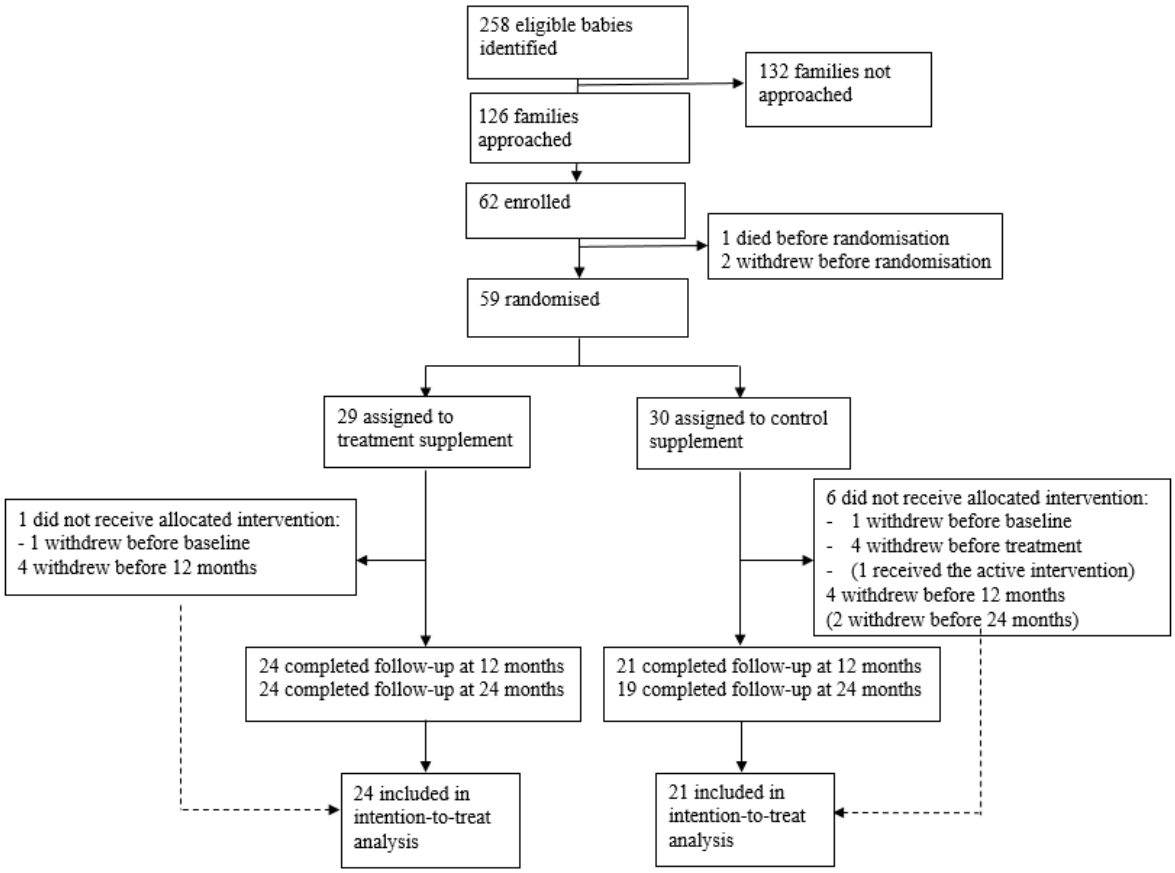
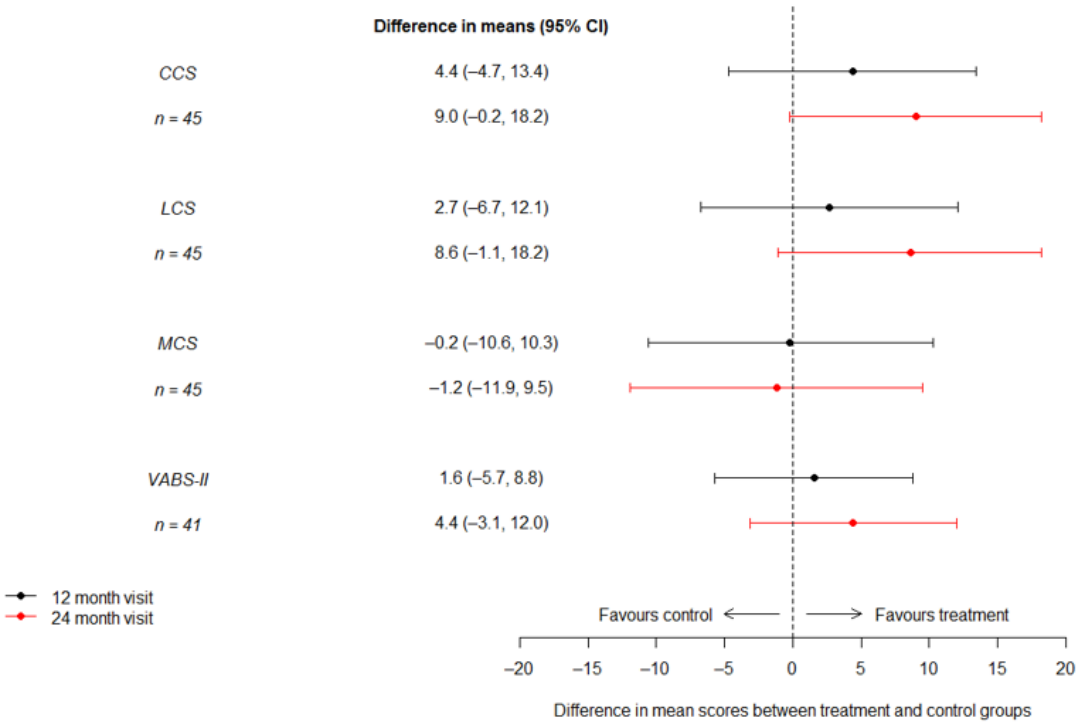


Figure 2: Adjusted mean difference in treatment and control groups by visit for the Bayley Scales of Infant Development, Third Edition, composite cognitive (CCS), language (LCS), and motor score (MCS), and Vineland Adaptive Behavior Scale, Second Edition (VABS-II), domain standard score. CI, confidence interval.



Mean difference in treatment and control groups by visit for the Bayley Scales of Infant Development III composite cognitive, language and motor score and Vineland Adaptive Behavior Scale II domain standard score.