Enteral supplementation with high-dose docosahexaenoic acid on the risk of bronchopulmonary dysplasia in very preterm infants: a collaborative study protocol for an individual participant data meta-analysis

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ABSTRACT
Introduction Severe bronchopulmonary dysplasia (BPD) is a well-known factor consistently associated with impaired cognitive outcomes. Regarding reported benefits on long-term neurodevelopmental outcomes, the potential adverse effects of high-dose docosahexaenoic acid (DHA) supplementation on this short-term neonatal morbidity need further investigations in infants born very preterm. This study will determine whether high-dose DHA enteral supplementation during the neonatal period is associated with the risk of severe BPD at 36 weeks' postmenstrual age (PMA) compared with control, in contemporary cohorts of preterm infants born at less than 29 weeks of gestation.

Methods and analysis As part of an Australian–Canadian collaboration, we will conduct an individual participant data (IPD) meta-analysis of randomised controlled trials targeting infants born at less than 29 weeks of gestation and evaluating the effect of high-dose DHA enteral supplementation in the neonatal period compared with a control. Primary outcome will be severe grades of BPD (yes/no) at 36 weeks' PMA harmonised according to a recent definition that predicts early childhood morbidities. Other outcomes will be survival without severe BPD, death, BPD severity grades, serious brain injury, severe retinopathy of prematurity, patent ductus arteriosus and necrotising enterocolitis requiring surgery, sepsis, combined neonatal morbidities and growth. Severe BPD will be compared between groups using a multivariate generalised estimating equations log-binomial regression model. Subgroup analyses are planned for gestational age, sex, small-for-gestational age, presence of maternal chorioamnionitis and mode of delivery.

Ethics and dissemination The conduct of each trial was approved by institutional research ethics boards and written informed consent was obtained from participating parents. A collaboration and data sharing agreement will be signed between participating authors and institutions. This IPD meta-analysis will document the role of DHA in nutritional management of BPD. Findings will be disseminated through conferences, media interviews and publications to peer-reviewed journals.

STRENGTHS AND LIMITATIONS OF THIS STUDY
⇒ This protocol was developed in collaboration with a multidisciplinary team including experts in harmonisation of databases.
⇒ Large high-quality contemporary multicentre randomised controlled trials will be included, although their expected number is limited.
⇒ The primary outcome will be harmonised to reflect contemporary modes of respiratory support and to be correlated with the risk of long-term neurodevelopmental morbidities in very preterm infants.
⇒ An individual participant data meta-analysis will allow to perform analysis according to meaningful subgroups.

INTRODUCTION
Bronchopulmonary dysplasia (BPD) affects about half of preterm infants born at less than 29 weeks of gestation.1 Neonatal nutritional support is of great interest for the prevention of inflammation-related complications of prematurity, such as BPD, with the overall aim to optimise survival and long-term clinical outcomes. However, controversy remains on whether high-doses of the long-chain polyunsaturated fatty acid (LCPUFA) docosahexaenoic acid (DHA) should be added to the diet of the most premature infants.

Initially introduced to supplement formula at doses that mimic DHA levels in breast diet of the most premature infants.
milk, it has been suggested that supplementation with high-dose DHA may have a beneficial effect on neurodevelopment in infants born very preterm. Enteral supplementation with high-dose DHA during the neonatal period at a level that mimics in utero accretion has been associated with specific changes in cerebral growth and function following DHA accumulation in the brain of the infant born very preterm. Recent trials have consistently reported that high-dose DHA over the standard of care improved long-term neurodevelopment in infants born at less than 29 weeks of gestation. Such evidence of the long-term impact of a nutritional intervention provided in the neonatal period on neurodevelopment is of great value.

Besides these promising results on neurodevelopment, high-dose DHA was pointed out as a good candidate to decrease the risk of BPD in infants born very preterm. Indeed, DHA contributes to the modulation of inflammation and oxidative stress, both involved in the development of BPD in this high-risk population. However, our recent systematic review and meta-analysis concluded that enteral supplementation with high-dose DHA in the neonatal period was not associated overall with a decrease in BPD rate in infants born at less than 29 weeks of gestation. Moreover, combining the trials using a more stringent BPD definition based on pulse oximetry at 36 weeks’ postmenstrual age (PMA) to assess the need for supplemental oxygen and/or respiratory support (ie, physiological BPD), DHA was associated with an increased risk of BPD (two studies, relative risk (RR) 1.20 (95% CI 1.01 to 1.42)) compared with control. In addition, the meta-analysis suggested a possible but not significant association between DHA and severe BPD defined according to the National Institute of Child Health and Human Development (three studies, RR 1.17 (95% CI 0.97 to 1.41)). Based on such findings, concerns remain about administration of high-dose DHA to improve neurodevelopment given a potential association with severe BPD, which is a well-established risk factor for adverse cognitive outcomes.

Although mechanisms by which DHA improves neurodevelopment could be different from those involved in the development of BPD, we cannot exclude that heterogeneity in BPD definitions might also be in part responsible for the discrepancy in the results. In addition, the definitions of BPD and BPD severity have changed over time along with practices in neonatal care to take into account the introduction of modern respiratory management options, leading to variations in BPD incidence and its capacity to predict long-term outcomes. A new BPD severity classification according to modern modes of respiratory support administered at 36 weeks’ PMA, regardless of supplemental oxygen, was recently developed by Jensen et al in a large North American contemporary cohort of children born very preterm between 2011 and 2015. Taking into account the introduction of modern respiratory management options, this pragmatic definition supports the evidence of a relationship between more severe BPD at 36 weeks’ PMA and long-term neurodevelopmental and respiratory morbidities in infants born very preterm.

Therefore, based on the previous meta-analysis findings and a known association between more severe BPD and unfavourable neurodevelopmental outcomes, a deeper understanding of the association between DHA and severe BPD needs further investigations. Harmonisation of the severe BPD definition in the recent DHA trials according to modern criteria will strengthen the results and allow their interpretation in balance with the potential efficacy of DHA on long-term neurodevelopmental outcomes. Moreover, inconsistent differential responses of DHA on BPD were previously reported according to subgroups such as sex, gestational age and mode of delivery and need to be further explored in this more vulnerable population.

Objectives

This study will aim to determine whether high-dose DHA enteral supplementation during the neonatal period is associated with the risk of severe BPD at 36 weeks’ PMA compared with control, in contemporary cohorts of preterm infants born at less than 29 weeks of gestation. The association between high-dose DHA and severe BPD will also be explored in important subgroups according to sex, gestational age, small-for-gestational age, presence of maternal chorioamnionitis and mode of delivery.

METHODS AND ANALYSIS

Protocol and registration

This protocol has been registered to PROSPERO (CRD42023431063) and ClinicalTrials.gov (NCT05915806).

Design

We will conduct a one-stage individual participant data (IPD) meta-analysis as a secondary analysis of a systematic review we previously performed. The prior systematic review included a meta-analysis of aggregated data of trials that examined the effects of enteral supplementation with high-dose DHA during the neonatal period on the risk of BPD (any definition), death, BPD severity (any definition) and a combined outcome of BPD or death in preterm infants born less than 29 weeks of gestation. Information on data sources (searched up to 1 August 2022), search strategy, selection process, data extraction and risk of bias assessment was detailed in the traditional systematic review protocol and publication. The systematic review and traditional meta-analysis identified four randomised clinical trials (RCTs) that included 2304 preterm infants born at less than 29 weeks of gestation. All four trials included in the prior systematic review will be considered for inclusion in this IPD meta-analysis. Two authors (IM and EP) will independently assess each trial for eligibility according to the predefined following eligibility criteria of this IPD meta-analysis.
Eligibility criteria for trials to be included in the IPD meta-analysis

Trials included in our prior systematic review and traditional meta-analysis will be eligible for this IPD meta-analysis if they were registered RCTs of infants born preterm at less than 29 weeks of gestation and with adequate levels of blinding and allocation concealment. Moreover, eligibility will be restricted to trials conducted in a population of infants born after 2010 receiving contemporary respiratory care, similar to Jensen et al’s cohort within which the severity-based definition of BPD was developed.

The intervention has to involve enteral administration of high-dose DHA supplementation during the neonatal period. A high-dose DHA supplementation is defined as direct enteral DHA supplementation at a dose of at least 40 mg/kg/day or DHA supplementation of breast milk or formula aiming for at least 0.4% of total fatty acids. The intervention should be randomly assigned as either enteral administration of high-dose DHA supplementation OR a control with no or low-dose DHA. Trials evaluating intravenous DHA interventions or combined interventions (eg, DHA combined to other nutrients or LCPUFA) are not considered for inclusion in this IPD meta-analysis to isolate the DHA effects and avoid heterogeneity in the intervention.

The IPD meta-analysis will be conducted using a harmonised severity-based definition of BPD in eligible trials. This definition will be based on Jensen et al’s criteria that adequately predict childhood outcomes in a contemporary cohort of infants born very preterm. To be included, prospectively collected data from eligible trials should allow BPD severity outcome classification and harmonisation according to Jensen et al’s severity-based BPD criteria at 36 weeks’ PMA.

OUTCOMES

Primary outcome

The primary outcome for this IPD meta-analysis will be ‘severe BPD at 36 weeks’ PMA’ (yes/no) and was a priori defined based on a team consensus. BPD severities are defined on grades (ie, no BPD, grade 1, 2, 3 BPD) based on the mode of respiratory support administered at 36 weeks’ PMA, regardless of prior or current oxygen therapy according to Jensen et al’s criteria (table 1).

Infants will be classified as severe BPD (yes) if they presented with a ‘grade 2 or 3 BPD’ at 36 weeks’ PMA, the two most severe grades of BPD according to Jensen et al’s classification. Grade 2 is defined as respiratory support at 36 weeks’ PMA with nasal cannula ≥2 L/min (‘low’ flow) or non-invasive positive airway pressure (including nasal intermittent positive pressure ventilation or nasal continuous positive airway pressure). Grade 3 is defined as use of invasive mechanical ventilation at 36 weeks’ PMA.

Infants will be classified as not severe BPD (no) if they presented with ‘no BPD or grade 1 BPD’ at 36 weeks’ PMA according to Jensen et al’s classification. No BPD is defined as no support at 36 weeks’ PMA with nasal cannula ≤2 L/min (‘low’ flow).

Other respiratory outcomes

Further analyses will be performed using a binary composite outcome defined as ‘grade 2 or 3 BPD or death’ at 36 weeks’ PMA (yes/no). We will also perform an analysis on the ordinal variable referring to the severity grades of BPD, that is, no BPD, grade 1, 2 or 3 BPD at 36 weeks’ PMA according to Jensen et al’s criteria as previously described (table 1).

Other outcomes

Unless otherwise specified, the following other secondary outcomes will be defined up to 40 weeks’ PMA, the expected date of delivery or discharge home, whichever occurred first:

► Death from any cause at 36 weeks’ PMA.
► Serious brain injury defined as intraventricular haemorrhage of grade 3 or 4 (according to Papile et al’s) or periventricular leucomalacia.
► Severe retinopathy of prematurity (ROP) defined as unilateral or bilateral ROP of stage 4 or 5 (according to the International Committee on ROP) or any stage of ROP requiring any treatment.
► Neonatal morbidity count adapted from Schmidt et al, including ‘grade 2 or 3 BPD’, serious brain injury and severe ROP. A score from 0 to 3 will be attributed according to the presence or absence of each morbidity.

Growth up to 36 weeks’ PMA.
Necrotising enterocolitis requiring surgery.
Culture-proven sepsis.
Growth up to 36 weeks’ PMA.

Obtaining IPD
On the early period of identifying/selecting studies for the previous systematic review, the principal investigator (IM) has contacted the primary author of trials for potential agreement to share de-identified IPD from their trial for the purpose of this participant-level meta-analysis. An agreement for data sharing and collaboration will be signed including all research parties involved and their institutions.

Dataset harmonisation
The collaborators will develop a common data form and coding sheet before data harmonisation and analysis to request necessary data for IPD meta-analysis according to a data dictionary (variables, definition, coding, field type and unit of each variable). Data harmonisation will aim (1) to report characteristics and methods of each trial including recruitment, retention and adherence to the intervention; (2) to describe the population including maternal and infant characteristics; (3) to harmonise outcomes including the primary outcome according to common definitions prespecified in the current protocol. All IPD datasets will be harmonised and combined into one large dataset. Data harmonisation within studies will be performed by Maelstrom Research.20 Briefly, they will create a DataSchema of the variables (based on common data form and variables of interest for analysis) and assess the potential for harmonisation between studies. Furthermore, Maelstrom Research will prepare the script for harmonisation of the data and assess the final quality of the harmonisation. Data will be stored on a secure server with controlled access hosted by the Université Laval. Data and harmonisation quality will be assessed by Maelstrom Research by generating variable distribution and comparing data content across trial-specific datasets.

Statistical analyses
The one-stage approach for conducting IPD meta-analysis will analyse the individual data from trials together in a single statistical analysis using appropriate models. The statistician will be blinded to the intervention groups (groups A and B). The analyses will be conducted using SAS V9.4 (SAS Institute) with a two-sided significance level set at p≤0.05.

All participants included in the IPD meta-analysis will be analysed according to the group of randomisation (intention-to-treat). The unit of analysis will be the infants and all infants will be included.

Primary outcome: severe BPD (yes/no, that is, ‘grade 2 or 3 BPD’/‘no BPD or grade 1 BPD’) incidence will be compared between groups using a multivariate generalised estimating equations (GEE) log-binomial regression model to account for the clustering of multiple birth infants. The trials will be entered in the model as fixed effect accounting for the expected small number of studies.21 The independent or exchangeable working correlation matrix structure for each GEE model will be chosen with minimal value of the quasi-likelihood information criterion. Differences between treatment and control groups will be expressed as RR with a 95% CI adjusted for trials, study sites, sex and gestational age at birth.

Double interactions of treatment with (1) infant sex, (2) gestational age at birth (<27 weeks and 27–29 weeks of gestation), (3) small-for-gestational age (defined as a birth weight <10th percentile for sex and gestational age),22 (4) presence of maternal chorioamnionitis and (5) delivery mode (vaginal vs caesarean delivery) will be tested in separate GEE log-binomial models. All significant double interaction terms will be tested in a final single model and the backward selection method will be used to eliminate any non-significant interactions. In case of significant interaction, the GEE log-binomial model will be fitted in the corresponding subgroups.

Further secondary outcome analyses will use GEE log-binomial regression models for binary outcomes or GEE linear regression models for continuous outcomes. Finally, GEE proportional-odds ordinal regression model will be used for analysis of the four grades of BPD severity, that is, no BPD, grade 1, 2 or 3 BPD at 36 weeks’ PMA.

In case of convergence issues that precluded fitting the GEE log-binomial regression models, we will use the GEE log-Poisson (or negative binomial model in case of overdispersion).

A small number of missing data on the primary outcome is expected and no missing data on the explanatory variables. If the number of missing data on the primary outcome is higher than expected (≥10%), we will verify if the data are missing at random given the observed data. In this case, multiple imputation using the PROC MI procedure with 200 burn-in iterations will be performed to generate imputed values for the four grades of BPD severity, that is, no BPD, grade 1, 2 or 3 BPD at 36 weeks’ PMA. Multiple imputation will be performed using fully conditional specification ordinal response logistic regression methods to generate 20 complete datasets. This model for multiple imputation will include (1) all variables included in the model analyses, (2) all variables significantly associated with this outcome, (3) all variables significantly associated with missing data on this outcome and (4) the number of multiple births (as a fixed-effect covariate) to take into account the cluster structure of the data.23 Then, the GEE log-binomial models described above will be fitted in the 20 complete datasets after dichotomisation of imputed data as severe BPD (yes; ‘grade 2 or 3 BPD’ or not severe BPD (no; ‘no BPD or grade 1 BPD’). Finally, the PROC MIANALYZE procedure and COMBCHI SAS-macro24 25 will be used to combine the results of the last modelling analyses and generate valid statistical inferences. Sensitivity analyses
will also be conducted by presenting the best-worst and worst-best case analyses.25

**Risk of bias**

The systematic review and traditional meta-analysis identified four RCTs that included 2304 infants born less than 29 weeks of gestation and examined the association between high-dose enteral DHA and respiratory outcomes.9 These studies were mostly contemporary multicentre trials with prospective detailed data collection on respiratory outcomes. However, the number of trials expected to be included in this IPD meta-analysis could be lower depending on whether they meet the IPD eligibility criteria and the possibility for the authors and institutions to collaborate and share IPD. Therefore, power could be limited by the availability of data, especially for the subgroup analyses.

Based on our previous systematic review and meta-analysis, none of the trials identified as potentially eligible evaluated the effect of DHA on BPD severity using Jensen et al’s severity-based BPD criteria. The strength of Jensen et al’s classification is to strongly establish that the more severe forms of BPD are correlated with the risk of long-term neurodevelopment and respiratory morbidities in infants born very preterm, although it is uncertain whether BPD has a causal role on this relationship. Although the data-driven development of this pragmatic classification would represent the current standard of care, it does not reflect the pathophysiological reasons for the need of respiratory support. However, a specific collection of data on the use of modern respiratory support at 36 weeks’ PMA may not be available in all four trials potentially eligible for this IPD meta-analysis. Moreover, the definition may not perform as well in units that do not have access to the most sophisticated levels of care, although the choice of a binary primary outcome combining the most severe forms of BPD, as proposed in this study, would facilitate the generalisability of the results.

By including only trials that have evaluated an intervention with DHA supplementation alone in this IPD meta-analysis, we will isolate the specific effect of DHA supplementation without risking an interaction with other LCPUFA or supplement.

Finally, the expertise of our multidisciplinary Canadian–Australian collaboration will facilitate harmonisation of key outcome measures and core data variables with the support of Maelstrom Research team.

**Patient and public involvement**

Patients or the public were not involved in the design of this protocol. There is no plan to involve patients or the public in the conduct, reporting or dissemination of this research.

**Ethics and dissemination**

The conduct of each trial was approved by institutional research ethics boards and written informed consent was obtained from participating parents in the original trials. No further consent from the parents will be obtained specifically for this IPD meta-analysis as no new data are collected and analyses are still in line with the original purpose of the trials. However, ethics approval for this IPD meta-analysis will be obtained from institutions of each included trial. A collaboration and data sharing agreement will be signed between participating authors and institutions. Data from each included RCT will be de-identified and no new data will be collected for this study. The role of LCPUFA on physiology, pathogenesis and management of BPD is not well understood and may differ according to gestational age, sex or prenatal conditions. This IPD meta-analysis will add to the results of our previous systematic review and meta-analysis on aggregated variables to further document this relationship.

We address an important issue where a finding that DHA does not increase the risk of severe BPD in the context of contemporary cohorts and modern BPD diagnostic criteria leaves the door open for further investigations of DHA benefits for long-term outcomes. The results will inform parents and healthcare professionals of the potential benefits or harms of high doses of DHA on the risk of BPD. We will communicate the results of this IPD meta-analysis through conferences, media interviews and publications to peer-reviewed journals. This IPD meta-analysis is expected to be conducted from July 2023 to March 2024.

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**Contributors** LM contributed to conception and design of the study, developed the criteria and the analysis plan and drafted the manuscript. PML, AJM, CTC, MG, JFG, LM, MB, IF and MM contributed to conception and design of the study and developed the criteria. DS, EP, AB, TRS and LM contributed to conception and design of the study, developed the criteria and the analysis plan. All authors revised the manuscript for important intellectual content and approved the final manuscript.

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