abstract

BACKGROUND AND OBJECTIVE: Omega-3 long chain polyunsaturated fatty acid (LCPUFA) exposure can be associated with reduced neonatal morbidities. We systematically review the evidence for the benefits of omega-3 LCPUFAs for reducing neonatal morbidities in extremely preterm infants.

METHODS: Data sources were PubMed, Embase, Center for Reviews and Dissemination, and the Cochrane Register of Controlled Trials. Original studies were selected that included infants born at <29 weeks' gestation, those published until May 2013, and those that evaluated the relationship between omega-3 LCPUFA supplementation and major adverse neonatal outcomes. Data were extracted on study design and outcome. Effect estimates were pooled.

RESULTS: Of the 1876 studies identified, 18 randomized controlled trials (RCTs) and 6 observational studies met the defined criteria. No RCT specifically targeted a population of extremely preterm infants. Based on RCTs, omega-3 LCPUFA was not associated with a decreased risk of bronchopulmonary dysplasia in infants overall (pooled risk ratio [RR] 0.97, 95% confidence interval [CI] 0.82–1.13, 12 studies, n = 2809 infants); however, when considering RCTs that include only infants born at ≥32 weeks' gestation, a trend toward a reduction in the risk of bronchopulmonary dysplasia (pooled RR 0.88, 95% CI 0.74–1.05, 7 studies, n = 1156 infants) and a reduction in the risk of necrotizing enterocolitis (pooled RR 0.50, 95% CI 0.23–1.10, 5 studies, n = 900 infants) was observed with LCPUFA.

CONCLUSIONS: Large-scale interventional studies are required to determine the clinical benefits of omega-3 LCPUFA, specifically in extremely preterm infants, during the neonatal period. Pediatrics 2014;134:120–134

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KEY WORDS omega-3, fatty acids, systematic review, preterm, infants, neonatal

ABBREVIATIONS
ALA—α-linolenic acid
BPD—bronchopulmonary dysplasia
CI—confidence interval
DHA—docosahexaenoic acid
DINO—Docosahexaenoic acid for the Improvement in Neurodevelopmental Outcome
GA—gestational age
IVH—intraventricular hemorrhage
LCPUFA—long-chain polyunsaturated fatty acid
NEC—necrotizing enterocolitis
PMA—postmenstrual age
RCT—randomized controlled trial
ROP—retinopathy of prematurity
RR—risk ratio

Dr Marc conceptualized and designed the protocol, screened the titles and abstracts, and retrieved articles for inclusion and quality, performed the analyses, drafted the initial manuscript, and approved the final manuscript as submitted; Ms Zhang critically reviewed the protocol, screened the titles and abstracts and retrieved articles for inclusion and quality, drafted the tables and figures, and reviewed the final manuscript as submitted; Dr Lavoie screened the articles for quality, revised the manuscript, participated in the redaction of the final manuscript, and reviewed the final manuscript as submitted; Dr Rhainds designed and conducted the systematic database search, reviewed the manuscript, and approved the final manuscript as submitted; and Dr Lacaze-Masmonteil reviewed the manuscript and approved the final manuscript as submitted.

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Despite major improvements in perinatal and neonatal care over the past few decades, extremely preterm infants continue to face high morbidity and mortality. In North America, 1 of 8 of these infants do not survive and ~15% of survivors suffer major long-term neurodevelopmental disabilities. In these vulnerable infants, the neonatal period is a critical time that can set the stage for lifelong morbidities, such as respiratory diseases and cognitive, motor, and behavioral impairments. During this time, preventive measures can have great and lasting impact.1-4

Bronchopulmonary dysplasia (BPD) is one of the most common and most serious complications occurring in extremely preterm infants.5 BPD occurs in ~45% of infants born at <29 weeks of gestation and clinically presents with persistent need for supplemental oxygen or respiratory support beyond the neonatal period.6 The disease is characterized by impaired pulmonary vascular and alveolar development. A dysregulation of inflammation has been proposed to play a central role in the etiology of lung injury characteristic of this disease. Inflammatory responses are well known to be modulated by long-chain polyunsaturated fatty acids (LCPUFAs), including omega-3 fatty acids, such as docosahexaenoic acid (DHA). It also has been proven that extremely preterm infants are deficient in LCPUFAs, particularly in DHA.6 Therefore, it is possible that supplementing the diet of extremely preterm infants with DHA could reduce inflammation that predisposes to BPD and other major neonatal diseases.6-10

Establishing a causal link between exposure to omega-3 lipids and neonatal outcomes in high-risk preterm infants has important clinical implications in neonatal care. Recent studies have investigated associations between reduced nutritional omega-3 during the perinatal or neonatal period and adverse neonatal outcomes, with the hope that supplementation may improve those outcomes.11-15 However, whether supplementing in omega-3 improves neonatal health in extremely preterm infants is unknown.

The objective of this study was to conduct a systematic review of observational studies and randomized controlled trials and, where possible, a meta-analysis, to examine whether omega-3 LCPUFA exposure or its supplementation (compared with no exposure or a placebo group) reduces the incidence of BPD and other major adverse neonatal outcomes in extremely preterm infants.

METHODS

The protocol of this review has been registered in PROSPERO (#CRD42013004794 http://www.crd.york.ac.uk/NIHR_PROSPERO/) with the participation of a multidisciplinary team, including neonatologists, epidemiologists, and research methodologists. We used the Preferred Reporting Items for Systematic Reviews and Meta-Analysis statement for reporting the review.14

Search Strategy and Study Selection:

We searched PubMed, Embase up to May 2013, and specialized sites (eg, Center for Reviews and Dissemination, Cochrane Register of Controlled Trials) by using MeSH terms and text words, adapting the search strategy if needed for each database. The search strategy on PubMed was as follows: #1 “Infant, Prematurity”[Mesh] OR “Infant, Very Low Birth Weight”[Mesh] OR (“preterm OR premature” AND (infant* OR newborn* OR neonat*)) #2: “Fish Oils”[Mesh] OR “fish oil” OR n-3 OR “omega-3” OR omega-3 OR PUFA OR “polyunsaturated fatty acid” OR “marine oil” OR “algal oil” OR “Docosahexaenoic Acids”[Mesh] OR “docosahexaenoic acid” OR DHA #3: inclusion of strategy #1 AND #2. In Embase, the strategy search used the terms: #1: ‘prematurity’/exp OR ‘very low birth weight’/exp OR (preterm OR premature AND (infant* OR newborn* OR neonat*)) #2: ‘fish oil’/exp OR ‘polyunsaturated fatty acid’/exp OR ‘omega-3 fatty acid’/exp OR ‘docosahexaenoic acid’/exp OR ‘fish oil’/pufa OR ‘polyunsaturated fatty acid’ OR ‘n-3’ OR ‘omega-3’ OR ‘marine oil’ OR ‘algal oil’ #3: #1 AND #2. We developed search strategies with terms related to the exposure/intervention (omega-3) and the target population (preterm) and did not use any filter for randomized controlled trials (RCTs) or cohort studies to maximize the sensitivity of the search strategies. There was no language restriction. Finally, we looked at reference lists and citations of relevant articles (previous reviews and included studies) to identify any additional eligible studies. All citations were then combined and duplicates were excluded. Studies were excluded if they were conference abstracts, reviews, or case reports. The bibliographies of review articles were manually searched for potentially relevant citations that were not detected by the electronic search.

Eligibility Criteria

We considered all observational studies (case-control, cohort, cross-sectional) or randomized controlled trials assessing the effects of omega-3 LCPUFA on mortality or adverse neonatal outcomes in infant populations including exclusively or in part extremely preterm infants. Extremely preterm infants were defined as infants with a gestational age (GA) of <29 weeks of gestation. For the purpose of this study, studies were included that reported any omega-3 LCPUFA exposure in the infant (eg,
measured on blood concentration) or any omega-3 LCPUFA supplementation directly to the infant in the neonatal period or through the mother during pregnancy or lactation. The intervention or exposure was compared with other standard interventions, placebo, or any other control levels of omega-3 LCPUFA exposure. The primary outcome of interest in this study was BPD-free survival at 36 weeks postmenstrual age (PMA), with BPD defined as the need for oxygen and/or ventilation at 36 weeks PMA. Nevertheless, studies reporting BPD with any definition were considered in the review. Studies also were included even if they did not include data on our primary outcome as long as they included other relevant secondary neonatal outcomes listed in the next sentence. Secondary outcomes (as defined in the original publications) comprised death; duration of ventilation or oxygen support; length of hospitalization; or occurrence of intraventricular hemorrhage (IVH), periventricular leukomalacia, necrotizing enterocolitis (NEC), infections, retinopathy of prematurity (ROP), or hemodynamically significant patent ductus arteriosus. Mortality was defined as death from any cause. Because these specific outcomes of interest might not have been reported in the title or the abstract, we did not narrow our initial search to include them. Instead, we did filter out databases to include any of these outcomes when reviewing articles at the stage of thorough reading.

Data Extraction Process

Two reviewers (P.Z., I.M.) independently screened the titles for potential relevance. Only titles that were mutually agreed as being not relevant were excluded. The 2 reviewers then screened abstracts and, finally, full-text articles for inclusion/exclusion criteria. To reach consensus, reviewers resolved disagreement between themselves by further discussion. Once the articles were selected for the purpose of full-text review, the 2 investigators independently reviewed the articles and extracted the following information by using a specific form: year and country of the study; study population; study design; type of the interventions or exposure (source of omega-3, dose, mode of administration, timing, association with another LCPUFA); comparison groups, such as a placebo or another nutrient or another dosage or mode of administration; number of exposed/unexposed or cases/controls; definitions and criteria used for BPD and each of the neonatal outcomes; and dichotomous variables or continuous data. The risk of bias was assessed by examining the sample selected, recruitment method, completeness of follow-up, and blinding according to the guidelines for assessing non-RCTs proposed by the Ohio Department of Mental Health and adapted in French by us (available at www.chuq.qc.ca/fr/evaluation/utmis). RCTs were assessed by the checklist proposed by COMPUS Adapted SIGN 50 (www.sign.ac.uk/methodology/checklists.html). To indicate the summary judgment of quality of each study, we used the summary ratings of unsatisfactory, satisfactory, or very satisfactory.

Definitions of Neonatal Outcomes

Neonatal outcomes were defined by the presence or absence of the diagnosis based on standard criteria or the criteria as reported in the studies. When >1 category was available for a given neonatal outcome (eg, none, mild, moderate, severe) and when these categories were mutually exclusive, we combined numbers to provide an effect estimate for “any definition” of the neonatal outcome. Otherwise, we extracted data matching with the more severe category of the outcome. If there were >2 comparison groups (eg, different sources of LCPUFA versus a placebo), we combined the data of the experimental groups if they involved similar intervention; furthermore, these data were compared with the data of the reference group (eg, placebo). Finally, in cases in which outcome data were available for subgroups of preterm infants as part of the larger studied group, only the data from the subgroup that was the closest to our population of interest (ie, preterm infants of <29 weeks’ gestation) were included in the combined analyses for this outcome. For example, results for the outcome BPD in the subgroup of infants with a birth weight <1250 g available from Manley et al 201111 were included in the meta-analysis instead of the results for the preterm infants of <33 weeks as reported in Makrides et al 2009.15

Synthesis of Results

We classified studies according to their design and also by neonatal outcomes. For dichotomous data, we presented results as summary risk ratio (RR) with 95% confidence intervals (CIs). Continuous data were not pooled, because they were measured only in a minority of studies. We used fixed-effect meta-analysis for combining data where it was reasonable to assume that studies were estimating the same underlying treatment effect (ie, where the trials’ population interventions and methods were judged sufficiently similar). If there was a clinical heterogeneity or if we detected substantial statistical heterogeneity, we used random-effects meta-analysis to produce an overall summary. Heterogeneity of the effect estimates was assessed by calculating the I² statistic,12 and significant heterogeneity was defined per protocol as I² ≥50%. Prespecified subgroup analyses were performed where possible for the infant GA (studies including preterm infants of <37 weeks versus studies targeting preterm infants of ≤32 weeks of gestation), the DHA dose,
timing and duration of exposure, the definition or severity of the outcomes, the mode of administration, and for the comparison between omega-3 provided alone or in association with another LCPUFA (ie, omega-6 LCPUFA).

RESULTS

Overview of Included Studies

Using our search strategy, we identified and screened 1876 citations. Fig 1 describes the process of selection for the final 24 studies included in the qualitative analysis. Characteristics of the 24 studies are summarized in Table 1. Eighteen of these studies were RCTs.\textsuperscript{11–13,15–29} Among nonrandomized studies, 4 were interventional studies with a control group\textsuperscript{30–35} and the remaining 2 studies\textsuperscript{8,34} did not test an intervention because they examined the association of BPD with LCPUFA concentrations in blood or in tracheal aspirates. Fifteen RCTs presented dichotomous outcomes. Finally, 13 studies (7 RCTs) assessed outcomes in a population of preterm infants of \( \leq 32 \) weeks of gestation. No RCT or study subanalyses were performed exclusively in extremely preterm infants (<29 weeks of gestation).

Characteristics of Exposure and Outcomes

In randomized and nonrandomized interventional studies, there was heterogeneity between studies regarding the sources of omega-3 (fish, alga, egg), their mode of administration (intravenous or enteral), the vehicle used for supplementation (formula or breast milk), the timing of exposure (started within 10 days after birth or later, but usually provided at least up to 36 weeks of PMA), the DHA percentage in formula or breast milk (usually <0.40% of total fatty acids [with high dose defined as DHA concentration of \( \sim 1\% \) of total fatty acids in the experimental group product]), or the cointerventions (exposure to omega-3 LCPUFA alone, generally DHA, or in association with other nutrients, usually in combination with arachidonic acid) (Table 1). Except for 1 study,\textsuperscript{30} the DHA intakes (dosage in mg/kg/d) based on the daily amount of feeds in the first weeks of life and the percentage of DHA in formula or breast milk were not reported. No RCTs reported survival free of BPD as an outcome. None of the studies reported BPD as a primary outcome. Most of the studies (\( n = 15 \), including 12 RCTs) defined BPD according to an oxygen requirement up to 28 days of postnatal age, or at 36 weeks PMA, but none of these studies used a physiologic definition\textsuperscript{35} (Table 2). Of all the observational studies or RCTs reporting neonatal outcomes as dichotomous variables, the association between omega-3 LCPUFA and any definition of BPD was examined in 18 studies (combined in 12 RCTs), with 1 RCT\textsuperscript{23} reporting a composite outcome including BPD. Similarly, NEC was reported in 14 studies (combined in 11 RCTs), IVH in 12 studies (combined in 7 RCTs), infections in 15 studies (combined in 10 RCTs), ROP in 10 studies (combined in 6 RCTs), and death in 8 studies (combined in 6 RCTs). Several studies examined >1 neonatal outcome at a time (Table 1). Only a few studies reported other relevant neonatal outcomes, such as number of days of ventilation, or the use or duration of supplementation oxygen,\textsuperscript{13,20,22,27,28} patent ductus arteriosus,\textsuperscript{12,20,23,30–32} cholestasis,\textsuperscript{31,32} or number of days of hospital admission.\textsuperscript{8,11,13,18,31,32}

Effect Estimates for Dichotomous Neonatal Outcomes

For each neonatal outcome of interest, combined effect estimates were calculated where possible and are outlined in Figs 2, 3, 4, and 5. Heterogeneity of effect estimates was reported.
<table>
<thead>
<tr>
<th>Studies</th>
<th>Participants</th>
<th>Control Group</th>
<th>Treatment Group</th>
<th>Timing</th>
<th>Dichotomous Outcomes (Any Definition) or Equivalent Continuous Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RCTs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>Carlson 1992, United States</td>
<td>65 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with marine oil</td>
<td>From enteral feeding &gt;110 kcal/kg/day (day 9 to day 40) to discharge</td>
<td>—</td>
</tr>
<tr>
<td>GA &lt; 32 wk, BW &lt; 1400 g</td>
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<td></td>
<td>GA</td>
</tr>
<tr>
<td>Carlson 1996, United States</td>
<td>94 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with marine oil</td>
<td>≤day 5 to discharge</td>
<td>—</td>
</tr>
<tr>
<td>GA &lt; 30 wk</td>
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<td></td>
<td></td>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>Carlson 1998, United States</td>
<td>120 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with egg phospholipids</td>
<td>~day 5 to discharge</td>
<td>✓</td>
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<tr>
<td>GA ≤ 32 wk, BW 725–1375 g</td>
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<td></td>
<td>GA</td>
</tr>
<tr>
<td>Clandinin 2005, Canada</td>
<td>195 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with either fish and fungal oil</td>
<td>≥day 14 of enteral intake (mean ~day 30) to hospital discharge</td>
<td>—</td>
</tr>
<tr>
<td>GA &lt; 35 wk or Algal oil</td>
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<td></td>
<td></td>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>Fewtrell 2002, United Kingdom</td>
<td>195 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with egg phospholipids</td>
<td>Day 10 to discharge neonatal unit</td>
<td>✓</td>
</tr>
<tr>
<td>GA &lt; 37 wk, BW &lt; 1750 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>Fewtrell 2004, United Kingdom</td>
<td>238 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with borage and fish oil</td>
<td>1st days of life (mean ~day 14) to 9 mo after term</td>
<td>✓</td>
</tr>
<tr>
<td>GA &lt; 35 wk, BW ≤ 2000 g</td>
<td></td>
<td></td>
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<td></td>
<td>GA</td>
</tr>
<tr>
<td>Foreman-van Drongelen 1996,</td>
<td>43 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with algal and fungal oils</td>
<td>&lt;day 10 to 3 mo</td>
<td>—</td>
</tr>
<tr>
<td>Netherlands</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>Groh-Wargo 2005, United States</td>
<td>60 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with either fish and fungal oil</td>
<td>Within 72 h of first enteral feeding, by day 28 to 1 yr</td>
<td>—</td>
</tr>
<tr>
<td>GA &lt; 33 wk, BW 750–1800 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GA</td>
</tr>
<tr>
<td>Studies</td>
<td>Participants</td>
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<tr>
<td>Harper 2010, United States</td>
<td>852 pregnant women with a history of previous preterm birth</td>
<td>Placebo mineral oil (no LCPUFA)</td>
<td>Mother daily omega-3 fish oil supplementation (1200 mg EPA and 800 mg DHA)</td>
<td>During pregnancy within 16–22 wk to 36 wk of gestation or delivery</td>
<td>— ✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Henriksen 2008, Norway</td>
<td>141 preterms</td>
<td>Daily infant supplementation with 0.5 mL control oil (no DHA, no ARA) /100 mL human milk (mother or donor)</td>
<td>Daily infant supplementation with 0.5 mL study oil (DHA 32 mg, ARA 31 mg) /100 mL human milk (mother or donor)</td>
<td>From enteral feeding &gt;100 mL/kg/d to ~day 65 post delivery</td>
<td>✓ ✓ ✓ ✓ ✓ —</td>
</tr>
<tr>
<td>Innis 2002, Canada</td>
<td>194 preterms</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with either alga oil (DHA alone) DHA not reported ARA 0.00%</td>
<td>From enteral feeding 50 kcal/kg/d for at least 28 d</td>
<td>— — ✓ — — ✓</td>
</tr>
<tr>
<td>Koletzko 1995, Germany</td>
<td>27 preterms</td>
<td>Formula without LCPUFA</td>
<td>Formula enriched with egg-TG and evening primrose oil DHA 0.30% EPA 0.03% ARA 0.50%</td>
<td>From full enteral feeding (≥130 mL milk/kg/d) for at least 21 d</td>
<td>— — ✓ — — —</td>
</tr>
<tr>
<td>Makrides 2010, Australia</td>
<td>2399 pregnant women &lt;21 wk of gestation</td>
<td>Daily supplementation with vegetable oil</td>
<td>Daily supplementation with omega-3 fish oil (100 mg EPA and 800 mg DHA)</td>
<td>During pregnancy from 21 wk of gestation until birth</td>
<td>✓ — — — — —</td>
</tr>
<tr>
<td>Manley 2011 substudy of Makrides 2009, Australia</td>
<td>657 preterms</td>
<td>Breast milk</td>
<td>Breast milk</td>
<td>Within 5 d of any enteral feeding to expected date of delivery</td>
<td>✓ ✓ ✓ ✓ ✓ ✓</td>
</tr>
</tbody>
</table>

EPA: 0.10% ARA: 0.43% or fish oil and egg triglycerides
DHA: 0.24% EPA: 0.00% ARA: 0.41%

EPA: 0.24% ARA: 0.41%

EPA: 0.00% ARA: 0.41%

EPA: 0.00% ARA: 0.41%

EPA: 0.10% ± 0.09 ARA: 0.08% ± 0.02

EPA: 0.18% ± 0.06% ARA: 0.43% ± 0.11%
<table>
<thead>
<tr>
<th>Studies</th>
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<th>Timing</th>
<th>Dichotomous Outcomes (Any Definition) or Equivalent Continuous Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Connor 2001, United States&lt;sup&gt;20&lt;/sup&gt;</td>
<td>470 preterms GA &lt; 30 wk BW &lt; 1800 g</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with fish and fungal oil: DHA 0.26%, EPA 0.08%, ARA 0.42% or egg-TG and fish oil: DHA 0.24%, EPA 0.00%, ARA 0.41%</td>
<td>Within 72 h of the first enteral feeding to term</td>
<td>— √ √ — — √</td>
</tr>
<tr>
<td>Skouroliakou 2010, Greece&lt;sup&gt;27&lt;/sup&gt;</td>
<td>38 preterms GA &lt; 32 wk BW &lt; 1500 g</td>
<td>Intravenous lipid emulsion containing conventional soybean lipid (Intralipid)</td>
<td>Intravenous lipid emulsion SMOFlipid, containing lipids from soybean oil (30%), olive oil (25%), and fish oil (15%) and medium-chain TG (30%)</td>
<td>From day 1–2 postbirth to at least day 14</td>
<td>— √ — — — —</td>
</tr>
<tr>
<td>Tomsits 2010, Hungary&lt;sup&gt;28&lt;/sup&gt;</td>
<td>60 preterms GA ≤ 34 wk BW 1000–2500 g</td>
<td>Intravenous lipid emulsion containing conventional soybean lipid (Intralipid 20%)</td>
<td>Intravenous lipid emulsion SMOFlipid, containing lipids from soybean oil (30%), olive oil (25%), and fish oil (15%) and medium-chain TG (30%)</td>
<td>From day 3–7 postbirth to day 14</td>
<td>— √ — — — √</td>
</tr>
<tr>
<td>Vanderhook 1999, United States&lt;sup&gt;29&lt;/sup&gt;</td>
<td>288 preterms BW 750–2000 g</td>
<td>Standard formula (no LCPUFA)</td>
<td>Formula enriched with fish oil: DHA 0.35%, EPA not reported, ARA 0.50%</td>
<td>Approximately up to 3–4 wk before full enteral feeding until 48 wk postconceptional age</td>
<td>— √ — — — √</td>
</tr>
<tr>
<td>Observational studies and interventional clinical trials.</td>
<td></td>
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</tr>
<tr>
<td>Marc 2011, Canada&lt;sup&gt;30&lt;/sup&gt;</td>
<td>36 preterms GA ≤ 29 wk</td>
<td>Breast milk</td>
<td>Breast milk: DHA 1.20% ± 0.60%, EPA 0.00% ± 0.10%, AA 0.40% ±0.20%</td>
<td>From day 3–7 post birth to 36 wk PMA</td>
<td>√ √ √ — √</td>
</tr>
<tr>
<td>Martin 2011, United States&lt;sup&gt;31&lt;/sup&gt;</td>
<td>88 preterms GA &lt; 30 wk</td>
<td>None</td>
<td>Fatty acids profiles</td>
<td>First day of birth to 1 mo postnatal</td>
<td>— √ √ √ √ √</td>
</tr>
<tr>
<td>Pawlik 2011, Poland&lt;sup&gt;32&lt;/sup&gt;</td>
<td>337 preterms GA &lt; 32 wk</td>
<td>Intravenous lipid emulsion containing soybean and olive oil emulsion (20% Clinoleic)</td>
<td>Intravenous lipid emulsion containing 50% of soybean and olive oil emulsion (20% Clinoleic)</td>
<td>Day 1 after birth to relay with enteral nutrition</td>
<td>— √ — √ √ √</td>
</tr>
</tbody>
</table>
Although most of the nonrandomized interventional studies were in favor of the intervention, they were not pooled because of the clinical and statistical heterogeneity between the studies with regard to design, DHA exposure, and methodology (Table 1).

Among RCTs reporting the association between omega-3 LCPUFA and BPD (n = 12), the between-study variance (3%) for鱼类 fatty acids was not significant total fatty acids. None of the 12 RCTs reported an increased risk of BPD in the experimental group. Four-point estimates yielded RRs, 1, 3 of which had CIs that extended above 1.

As part of a large study including premature infants born at 42 to 44 weeks of gestation and delivered early after birth to the preterm infants through breast milk from their mothers, who had been supplemented with DHA during their lactation period. According to these data, a nearly significant lower risk for the development of BPD (adjusted RR 0.75; 95% CI 0.57–0.98) was found in subgroup analyses of the 5 RCTs targeting only the preterm infants born at 32 weeks of gestation or less. None of the 12 RCTs reported an increased risk of BPD in the experimental group. Four-point estimates yielded RRs, 1.3 of which had CIs that extended above 1.

The RR of NEC reported by the 5 RCTs reporting the effects of omega-3 LCPUFA in preterm infants was 44% of the total variance (Fig 3). With the exception of 2 studies with RRs > 1, all other studies had RRs < 1.0. The RR of NEC reported by the 5 RCTs reporting the effects of omega-3 LCPUFA in preterm infants was 44% of the total variance (Fig 3). With the exception of 2 studies with RRs > 1, all other studies had RRs < 1.0. The RR of NEC reported by the 5 RCTs reporting the effects of omega-3 LCPUFA in preterm infants was 44% of the total variance (Fig 3).

### Table 1

<table>
<thead>
<tr>
<th>Studies</th>
<th>Participants</th>
<th>Control Group</th>
<th>Treatment Group</th>
<th>Timing</th>
<th>Dichotomous Outcomes (Any Definition) or Equivalent Continuous Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Death</td>
<td>BPD</td>
</tr>
<tr>
<td>Pawlik 2011, Poland</td>
<td>84 preterms</td>
<td>Intravenous lipid emulsion (10% Omegaven)</td>
<td>Intravenous lipid emulsion 50% fish-oil emulsion (Clinoleic 20%)</td>
<td>Day 1 after birth to relay with enteral nutrition</td>
<td>--</td>
</tr>
<tr>
<td>Rudiger 2000, Germany</td>
<td>25 preterms</td>
<td>None</td>
<td>Tracheal and pharyngeal amount of PUFAs</td>
<td>Day 1 after birth and in the following 4 d</td>
<td>--</td>
</tr>
<tr>
<td>Skoufakis 2012, Greece</td>
<td>282 preterms</td>
<td>Parenteral lipid emulsions of soybean-lipid</td>
<td>Parenteral lipid emulsions of medium-chain TG and omega-3–PUFA (SMOFlipid)</td>
<td>First or second day after birth for at least 7 d</td>
<td>√</td>
</tr>
</tbody>
</table>

√ reported outcomes; ARA, arachidonic acid; BW, birth weight; CRI, corrected age; EPA, eicosapentaenoic acid; PUFAs, polyunsaturated fatty acids; TG, Triglycerides.
TABLE 2 The Reporting of BPD

<table>
<thead>
<tr>
<th>Studies</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carlson 199216</td>
<td>No definition for BPD</td>
</tr>
<tr>
<td>Carlson 199817</td>
<td>Supplemented oxygen for &gt;28 d and/or pulmonary changes on radiologic examination</td>
</tr>
<tr>
<td>Carlson 199818</td>
<td>Supplemental oxygen on day 28 of age with radiographic changes</td>
</tr>
<tr>
<td>Clandinin 200518</td>
<td>Supplemental oxygen at 36 wk PMA with severe or chronic radiologic changes on chest x-ray</td>
</tr>
<tr>
<td>Fewtrell 200220</td>
<td>Oxygen requirement &gt;30% for &gt;28 d</td>
</tr>
<tr>
<td>Fewtrell 200421</td>
<td>Respiratory assistance (ventilation, CPAP, or supplemental oxygen) at 36 wk</td>
</tr>
<tr>
<td>Foreman-Van Drongelen 199627</td>
<td>No definition for BPD</td>
</tr>
<tr>
<td>Groh-Warg0 200523</td>
<td>Composite outcomes (Need for supplemental oxygen beyond 1 mo postnatal or 36 wk corrected age (CA) OR patent ductus arteriosus OR IVH)</td>
</tr>
<tr>
<td>Harper 201012</td>
<td>Requirement for supplemental oxygen at 36 wk CA for neonates born before 34 wk</td>
</tr>
<tr>
<td>Henriksen 200825</td>
<td>Need for respiratory support (no mention of timing); days with oxygen</td>
</tr>
<tr>
<td>Manley 201111</td>
<td>Oxygen required at 36 wk PMA</td>
</tr>
<tr>
<td>O’Connor25 2001</td>
<td>Supplemental oxygen beyond 1-mo postnatal age or at 36 wk PMA</td>
</tr>
<tr>
<td>Skouroliakou 201037</td>
<td>Days of ventilation (no dichotomous variable was reported)</td>
</tr>
<tr>
<td>Tomstsi 201028</td>
<td>Days of supplemental oxygen (no dichotomous variable was reported)</td>
</tr>
<tr>
<td>Vanderhoof 199929</td>
<td>No definition for BPD</td>
</tr>
<tr>
<td>Marc I 201110</td>
<td>Supplemental oxygen required at 36 wk PMA</td>
</tr>
<tr>
<td>Martin 201116</td>
<td>Supplemental oxygen required at 36 wk PMA</td>
</tr>
<tr>
<td>Pawlik 201111</td>
<td>Oxygen requirement and/or respiratory support at 36 wk CA</td>
</tr>
<tr>
<td>Pawlik 201112</td>
<td>Oxygen requirement and/or respiratory support at 36 wk CA</td>
</tr>
<tr>
<td>Rudiger 200014</td>
<td>Need for mechanical ventilation or oxygen therapy at day 28 of age with radiologic signs of BPD</td>
</tr>
<tr>
<td>Skouroliakou 201221</td>
<td>Need for supplementary oxygen at day 28 of age and classified as mild, moderate, and severe according to the need for supplemental oxygen at 36 wk PMA or at 56 d postnataally</td>
</tr>
</tbody>
</table>

CA, corrected age; CPAP, continuous positive airway pressure.

Quality Analysis

Heterogeneity in study quality, design, and reporting made quality evaluation difficult, especially for some RCTs. The inclusion/exclusion criteria often excluded more at-risk populations. Randomization process was poorly reported in 5 RCTs. Depending on the report of randomization, percentage of follow-up loss for the main outcome, and blinding, the study quality was judged as unsatisfactory, satisfactory, or very satisfactory for 5, 7, and 6, respectively, of the 18 RCTs. Definition of the various neonatal outcomes and timing of measures according to the beginning of the intervention varied substantially across studies. Analyses were often not reported to be performed in intention-to-treat. The number of dropouts and the reasons for subjects lost to follow-up were not clearly reported. The 6 observational studies were classified as satisfactory.

DISCUSSION

The main finding of this study is the potential protective effect of omega-3 LCPUFA supplementation on BPD and NEC when examining RCTs exclusively targeting preterm infants born at ≤32 weeks of gestation. This finding is of major clinical relevance mainly because infants born at the extreme lower end of prematurity are at particularly high risk for a nutritional deficit in omega-3 lipids, potentially predisposing to adverse neonatal outcomes. These results also are in accordance with observational studies, indicating an association between DHA levels and adverse neonatal outcomes in very preterm infants.8,30,33

Preterm infants are nutritionally deficient in DHA. Daily nutritional DHA requirements of extremely preterm infants are estimated at the range of 40 to 60 mg/kg.36 An essential nutrient, the α-linolenic acid (ALA) is the major n-3 fatty acid. In the body, ALA is metabolized

born at ≤32 weeks of gestation, was in favor of the intervention, although this relative risk was not statistically significant (RR 0.50, 95% CI 0.23–1.10; I² = 0%). Nevertheless, the effect of administering omega-3 to these preterm infants was significantly different and more beneficial where the intervention focused on this population rather than on the older neonate population.

There was no clear trend in risk of ROP (n = 6 RCTs, RR 1.10, 95% CI 0.91–1.33; I² = 0%), infections (n = 10 RCTs, RR 1.10, 95% CI 0.91–1.33; I² = 0%), and IVH of any grade (n = 7 RCTs, RR 0.96, 95% CI 0.76–1.21; I² = 0%) between the preterm infants receiving a placebo or omega-3 LCPUFA-supplemented nutrition overall, or among subgroups according to GA analyses (Fig 4A and Fig 5). When taking into consideration the severity of IVH (grade 3–4; n = 5 studies; RR 1.37, 95% CI 0.67–2.81; I² = 0%) or the severity of the ROP (grade 3–4 or need for treatment; n = 3 studies; RR 0.86, 95% CI 0.47–1.58; I² = 0%), results did not change significantly.

Reporting of Continuous and Other Dichotomous Outcomes

Five studies individually reported no differences in the numbers of days of hospital admission (Table 1). A few studies reported either continuous or dichotomous outcomes relating to the neonatal outcomes of interest. The data available were for (1) BPD severity (1 observational study), (2) days of ventilation or with oxygen, (3) occurrence of periventricular leukomalacia (3 studies), and (4) type of infections (systemic, confirmed). The study results did not reach statistical significance. Pooling was not performed because of the small numbers of studies for these outcomes.

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to either eicosapentaenoic acid or DHA. As an important constituent of cell membranes, DHA is highly concentrated in several tissues, including the brain (23% of total body stores in a term infant), muscles, and stored adipose tissue. In extremely preterm infants, DHA stores represent <10% of the total body stores in lipids when compared with a full-term neonate. Preterm infants can synthesize small amounts of DHA from the omega-3 dietary precursor.

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**FIGURE 2**

Meta-analysis comparing the effects of LCPUFA supplementation on BPD. A, According to all RCTs and a subgroup of RCTs including preterm infants of ≤32 weeks of gestation. B, According to a subgroup of RCTs evaluating an intervention that exclusively supplement in omega-3 LCPUFA.
ALA, via the desaturation-elongation pathway. However, because of limited fat stores, considerable oxidation, and increased requirements to support rapidly growing tissues, availability of DHA is insufficient during the neonatal period.

In extreme preterm infants, high systemic inflammatory biomarkers are measured in the earliest hours after birth and for extended periods of time during the neonatal period, particularly in infants exposed to mechanical ventilation and supplemental O2. Preterm infants also have a limited ability to counteract inflammatory and oxidative stress during the neonatal period. Sustained oxidative stress and inflammation are key factors contributing to the development of BPD as well as other neonatal complications. Dietary LCPUFA, particularly DHA and eicosapentaenoic acid are important modulators of inflammation. Considering the anti-inflammatory effect of DHA, and of its downstream metabolite eicosanoids, authors have argued that DHA deficits in preterm infants may have considerable, underestimated adverse effects during the neonatal period. In light of the strong association between elevated inflammation and both short-and long-term morbidities in extreme preterm infants, we postulate that a DHA supplementation or a decrease in the omega-6/omega-3 reduces the occurrence of these major adverse neonatal outcomes, including BPD. Of note, when specifically examining studies in which omega-6 lipids were coadministered with DHA, we detected no additional benefit on BPD compared with studies using DHA alone (Fig 2B), indicating a selective role of DHA or the ratio omega-6/omega-3.

**Biological Plausibility**

Interventions that modulate DHA levels have been shown to reduce pulmonary inflammation in animal models, although data are lacking in humans. Indeed, intravenous omega-3 lipids improve the redox potential of glutathione and reduce lung inflammation in the neonatal guinea pig model. Maternal DHA supplementation reduces inflammation in the neonatal mouse hyperoxia model of BPD. Reduced serum DHA during the first postnatal month was associated with increased need for supplemental oxygen at 36 weeks PMA (odds ratio 2.5, 95% CI 1.3–5.0). The ratio of n6-linoleic acid to n3-DHA in these infants was highly predictive of lung disease, confirming the importance of balanced ratios of LCPUFA and potential selective role of DHA in protecting against BPD. A balanced LCPUFA ratio was also protective against late-onset infections, providing an additional protective mechanism against adverse neonatal outcomes.

Altogether, our data strongly suggest that low DHA contributes to a dysregulation of inflammation, predisposing to major neonatal morbidities, and that restoring an adequate ratio in LCPUFA may prevent the adverse effects of an excessive inflammatory and oxidative stress exposure repeatedly associated with lung injury in extreme preterm infants. This nutritional approach has gained interest recently, since publication of the Docosahexaenoic acid for the Improvement in Neurodevelopmental Outcome (DINO) trial and of a secondary analysis in low birth weight infants suggesting an effect of omega-3 LCPUFA to reduce the need for oxygen at 36 weeks PMA in this population. The DINO trial substantially influences but does not dominate the meta-analytic findings. Therefore, since the last review in 2008, this review is valuable for placing the DINO trial results in a broader context, and to highlight the need for a definitive trial testing the impact of LCPUFA on neonatal morbidities.

**Limitations**

Although it is the aim of this review to estimate treatment effects with more precision, the major limitation with our systematic review and meta-analysis of RCTs is the low occurrence of reported events on the outcomes of interest. This is mainly due to the large GA range of preterm infants included in the studies, and especially to the inclusion of a large

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Experimental</th>
<th>Control</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Total</td>
<td>Events</td>
<td>Total</td>
</tr>
<tr>
<td>Carlson 1998</td>
<td>1</td>
<td>34</td>
<td>5</td>
<td>85</td>
</tr>
<tr>
<td>Fewtrell 2002</td>
<td>4</td>
<td>95</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Fewtrell 2004</td>
<td>0</td>
<td>122</td>
<td>1</td>
<td>116</td>
</tr>
<tr>
<td>Henriksen 2008</td>
<td>0</td>
<td>68</td>
<td>2</td>
<td>73</td>
</tr>
<tr>
<td>Makrides 2009-Manley 2011</td>
<td>9</td>
<td>322</td>
<td>6</td>
<td>335</td>
</tr>
<tr>
<td>Makrides 2010</td>
<td>3</td>
<td>1197</td>
<td>12</td>
<td>1202</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>1838</td>
<td>1911</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Heterogeneity: CH² = 8.87, df = 5 (P = .11); I² = 44%
Test for overall effect: Z = 0.89 (P = .37)

FIGURE 3
Meta-analysis comparing the effects of LCPUFA supplementation on death.
proportion of older preterm infants who are relatively lower risk of neonatal complications. Furthermore, in none of the studies were neonatal outcomes stated as the primary outcomes, with older studies mainly reporting neonatal complications as safety outcomes only. Finally, the studies varied widely in terms of methodology quality, sample size, DHA doses, source and administration protocol, and in the populations studied. Because the daily amount of feeds administered to infants was not reported in most studies, it is impossible to extrapolate a dose-effect

FIGURE 4
Meta-analysis comparing the effects of LCPUFA supplementation on (A) NEC and (B) ROP according to all RCTs and a subgroup of RCTs including preterm infants of ≤32 weeks of gestation.
of DHA (eg, in mg/kg body weight) required to achieve a therapeutic benefit.

**IMPLICATIONS AND CONCLUSIONS**

According to results of preclinical experimental animal studies, as well as observational studies in humans, DHA supplementation is safe and likely to be beneficial in extremely preterm infants. This systematic review highlights potential benefits of DHA in the prevention of BPD or NEC when specifically targeting the younger, most immature preterm infants immature preterm infants. No detrimental effect was reported with omega-3 LCPUFA in this age group. In current clinical practice, enteral supplementation remains the best strategy for optimizing fat absorption and bioavailability in extremely preterm infants. Future studies focused on an early supplementation of DHA are required to establish more definitively the clinical benefits relative to therapeutic effects of different methods of supplementation of DHA for extremely premature infants, with the prospect of reducing neonatal mortality and morbidities in this age group. Overall, our results support the need for definitive RCTs to examine the effectiveness of omega-3 LCPUFA versus placebo in reducing the occurrence of BPD and of other adverse neonatal complications in extremely preterm infants. Of note, a large RCT is ongoing testing the effect of a direct oral supplementation of extremely preterm infants with fish oil (N3RO: N-3 LCPUFA for improvement in Respiratory Outcomes; www.anzctr.org.au/#ACTRN, trial #12612000503820).

**FIGURE 5**

Meta-analysis comparing the effects of LCPUFA supplementation on (A) neonatal infection according to all RCTs and a subgroup of RCTs including preterm infants of ≤32 weeks of gestation, and (B) IVH.
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