

A systematic review about long-chain polyunsaturated fatty acid supplementation in very preterm newborns: outcomes and safety

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Revisão sistemática sobre ácidos gordos polinsaturados de cadeia longa nos recém-nascidos muito pré-termo: resultados e segurança

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Abstract

Background: Long-chain polyunsaturated fatty acid (LCPUFA) supplementation is crucial for normal growth and development of preterm infants.

Aim: This systematic review aimed to identify the effects of LCPUFA supplementation on the main health outcomes of preterm infants born under 32 weeks of gestational age (GA) and to determine if doses higher than the established safety threshold can be administered to preterm infants to improve health outcomes without causing harm.

Methods: A comprehensive literature search was conducted using Medline, Scopus, and Web of Science databases from October 2023 to February 2024, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. A total of 36 articles were included based on the following criteria: preterm infants under 32 weeks of gestational age; and enteral LCPUFA supplementation. The quality of these studies was evaluated using the Cochrane Collaboration revised tool and the Newcastle-Ottawa Scale (NOS). This systematic review was pre-registered in advance on the International Prospective Register of Systematic Reviews (PROSPERO: CRD42023461646).

Results: Cognitive and neurodevelopmental outcomes, such as full-scale intelligence quotient scores and behavioural acuity, were improved by supplementation with docosahexaenoic acid (DHA) and arachidonic acid (AA). Gastrointestinal outcomes benefited from DHA:AA supplementation, leading to a significant reduction in necrotizing enterocolitis incidence. Higher LCPUFA levels were associated with better visual acuity and a reduced incidence of severe retinopathy of prematurity. Respiratory outcomes revealed no significant reduction in bronchopulmonary dysplasia incidence, but there was a potential benefit in improving respiratory support needs and lowering oxygen requirements. Growth and anthropometric outcomes were better in infants fed with human milk, regarding LCPUFA supplementation. Lastly, biomarkers and

nutritional status outcomes, including plasma essential fatty acids, vitamin E status, and red blood cell fatty acid composition, showed overall benefits when human milk or formula was supplemented with LCPUFA. No uniform safety dose value for LCPUFA has been found.

Conclusions: LCPUFA supplementation was linked to significant improvements in cognitive and neurodevelopment, visual, gastrointestinal, respiratory, anthropometric and growth, and biomarkers and nutritional status outcomes.

Keywords: very preterm infants; LCPUFA supplementation; health outcomes; neurodevelopment; growth; systematic review.

Resumo

Introdução: A suplementação de ácidos gordos polinsaturados de cadeia longa (LCPUFA) é crucial para o crescimento e desenvolvimento normais dos bebés prematuros.

Objetivo: Esta revisão sistemática teve como objetivo identificar os efeitos da suplementação com LCPUFA nos principais resultados de saúde dos bebés prematuros nascidos com menos de 32 semanas de idade gestacional (IG) e determinar se doses superiores ao limite de segurança estabelecido podem ser administradas a bebés prematuros para melhorar os resultados de saúde sem efeitos adversos.

Métodos: Foi realizada uma pesquisa bibliográfica abrangente nas bases de dados Medline, Scopus e Web of Science de outubro de 2023 a fevereiro de 2024, seguindo as diretrizes Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). Foram incluídos 36 artigos com base nos seguintes critérios: prematuros com menos de 32 semanas de idade gestacional; e suplementação entérica de LCPUFA. A qualidade destes estudos foi avaliada através da ferramenta revista da Cochrane Collaboration e da Escala de Newcastle-Ottawa (NOS). Esta revisão sistemática foi previamente pré-registada no Registo prospetivo Internacional de Revisões Sistemáticas (PROSPERO: CRD42023461646).

Resultados: Os resultados cognitivos e de neurodesenvolvimento, como as pontuações de quociente de inteligência à escala real e a acuidade comportamental, foram melhorados pela suplementação com ácido docosahexaenóico (DHA) e ácido araquidónico (AA). Os resultados gastrointestinais beneficiaram da suplementação de DHA:AA, levando a uma redução significativa da incidência de enterocolite necrosante. Níveis mais elevados de LCPUFA foram associados a uma melhor acuidade visual e a uma redução da incidência de retinopatia grave da prematuridade. Os resultados respiratórios não revelaram uma redução significativa na incidência de displasia broncopulmonar, mas houve um potencial benefício na melhoria das necessidades de

suporte respiratório e na redução das necessidades de oxigénio. O crescimento e os resultados antropométricos foram melhores nos lactentes alimentados com leite humano, em relação à suplementação com LCPUFA. Por último, os biomarcadores e os resultados do estado nutricional, incluindo os ácidos gordos essenciais plasmáticos, o estado da vitamina E e a composição dos ácidos gordos dos glóbulos vermelhos, mostraram benefícios globais quando o leite humano ou a fórmula foram suplementados com LCPUFA. Não foi encontrado um valor uniforme de dose de segurança dos LPUFA.

Conclusões: A suplementação de LCPUFA foi associada a melhorias significativas no desenvolvimento cognitivo e neurológico, visual, gastrointestinal, respiratório, antropométrico e de crescimento, para além dos biomarcadores e dos resultados do estado nutricional.

Palavras-chave: prematuro moderado; suplementação de LCPUFA; resultados em saúde; neurodesenvolvimento; crescimento; revisão sistemática.

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List of Abbreviations

AA - Arachidonic Acid

BPD - Bronchopulmonary dysplasia

BW - Birth weight

CA - Corrected age

DHA - Docosahexaenoic acid

DHM - Donor human milk

EFAs - Essential fatty acids

EPA - Eicosapentaenoic acid

EN - Enteral nutrition

ESPGHAN - European Society for Paediatric Gastroenterology Hepatology and Nutrition

FSIQ - Full scale intelligence quotient

GA - Gestational age

HM - Human milk

LCPUFA - Long-chain polyunsaturated fatty acid

NEC - Necrotizing enterocolitis

N-3 - Omega-3

N-6 - Omega-6

PN - Parental nutrition

IQ - Intelligence Quotient

RCT - Randomized control trial

ROP - Retinopathy of prematurity

UNICEF - The United Nations Children's Fund

WHO - World Health Organization

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1. Background

1.1. Prematurity

Prematurity is defined as a preterm birth occurring before 37 completed weeks of gestation, or fewer than 259 days since the first day of a woman's last menstrual period ^(1, 2). Various levels of prematurity are categorized according to birth weight (BW), the primary classification, and with gestational age (GA) which is based on the length of pregnancy after the first day of the last menstrual period ⁽¹⁻³⁾. This different way of classifying is shown in Table 1.

Table 1. Preterm categories.

Birth Weight (BW)	
Low birth weight (LBW)	< 2 500 g
Very Low birth weight (VLBW)	< 1 500 g
Extremely Low birth weight (ELBW)	< 1 000 g
Gestational Age (GA)	
Term	≥ 37 weeks
Late preterm	34 to < 37 weeks
Moderate preterm	32 to < 34 weeks
Very preterm	28 to < 32 weeks
Extremely preterm	< 28 weeks

According with World Health Organization ⁽¹⁾.

The endorsement of this recommendation by the American College of Obstetricians and Gynaecologists and the Society for Maternal-Fetal Medicine promotes the utilization of gestational age ^(4, 5).

1.1.1 Epidemiology

According to the World Health Organization (WHO)'s 2022 data, it is estimated that 15 million infants are born preterm each year, making up to 5 to 18 % of all births. In 2020, an estimated 13.4 million babies were born prematurely, representing 1 in 10 babies “born too soon”, before 37 completed weeks of gestation ^(1, 6). Globally, as illustrated in Figure 1, the incidence of preterm birth in 2020 was approximately 10%, with a considerable range observed across regions. The lowest incidence was observed in Europe, at 5%, while the highest was observed in Africa, at 18% ^(1, 7). Furthermore, over 60% of preterm births occurred in South Asia and sub-Saharan Africa ⁽⁸⁾. Among these premature births, 85% occurred between 32 to 36 weeks of gestation, 10% between 28 to less than 32 weeks of gestation and 5% under 28 weeks of gestation ⁽⁹⁾.

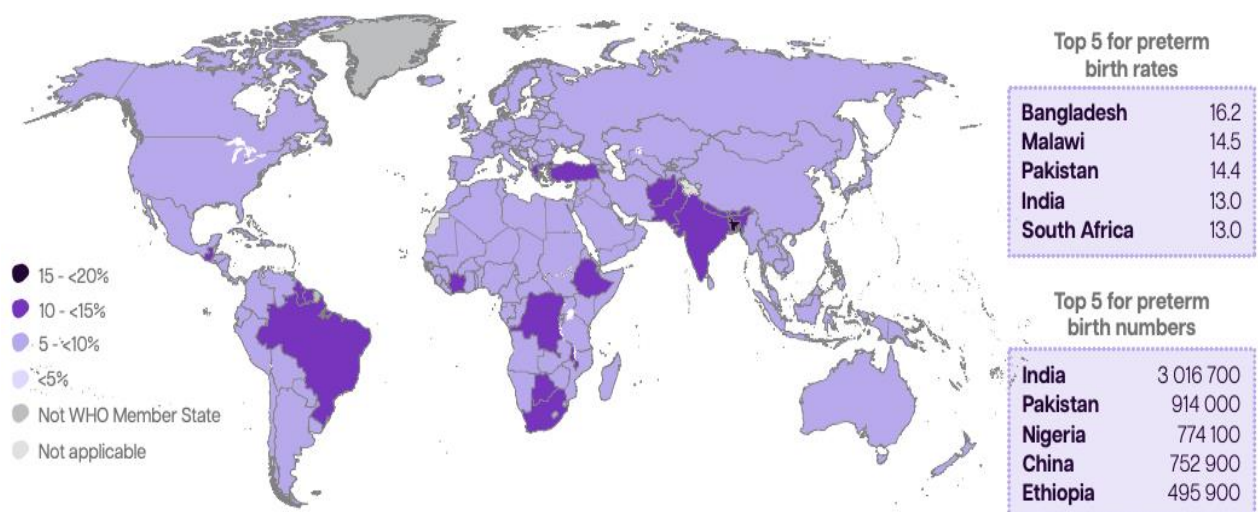


Figure 1. Estimated preterm birth rates by country for the year 2020

Image taken from World Health Organization, Born too soon: decade of action on preterm birth ^(6, 7).

The largest dataset employing a modelling approach estimated preterm birth rates from 2020, indicating a minimal global change from 2010 to 2020 (9.8% to 9.9% of live births). Regarding regional trends, preterm birth rates in southern Asia and sub-Saharan Africa, showed little to no change over the decade, despite the consistent

prevalence in sub-Saharan Africa where the total number of preterm births increased due to elevated fertility rates ⁽⁷⁾.

In 2020, as illustrated in Figure 1 and Figure 2, global data from the WHO and the United Nations Children's Fund (UNICEF) indicated significant disparities in preterm birth rates across regions. Southern Asia exhibited the highest preterm birth rate at 13.2%, followed by Sub-Saharan Africa (10.1%) ⁽¹⁰⁾. Conversely, eastern Asia, south-eastern Asia, Oceania, northern America, Australia, New Zealand, Central Asia, and Europe had rates below 8%. Notably, high-income countries such as Greece (11.6%) and the USA (10.0%) maintained relatively high preterm birth rates around 10% ^(6, 7, 10). Over half of all preterm births globally occurred in just eight countries in 2020: India, Pakistan, Nigeria, China, Ethiopia, Bangladesh, the Democratic Republic of the Congo, and the USA, attributed to large populations, high birth rates, and weaker health systems in these areas ^(6, 7, 10).

As reflected in Figure 2, Southern Asia recorded the highest number of preterm births at 4.8 million, including over 700,000 occurring before 32 weeks of gestation. Sub-Saharan Africa followed closely with 3.9 million preterm births, with 600,000 born before 32 weeks, posing higher risks of mortality and long-term health outcomes ⁽¹⁰⁾.

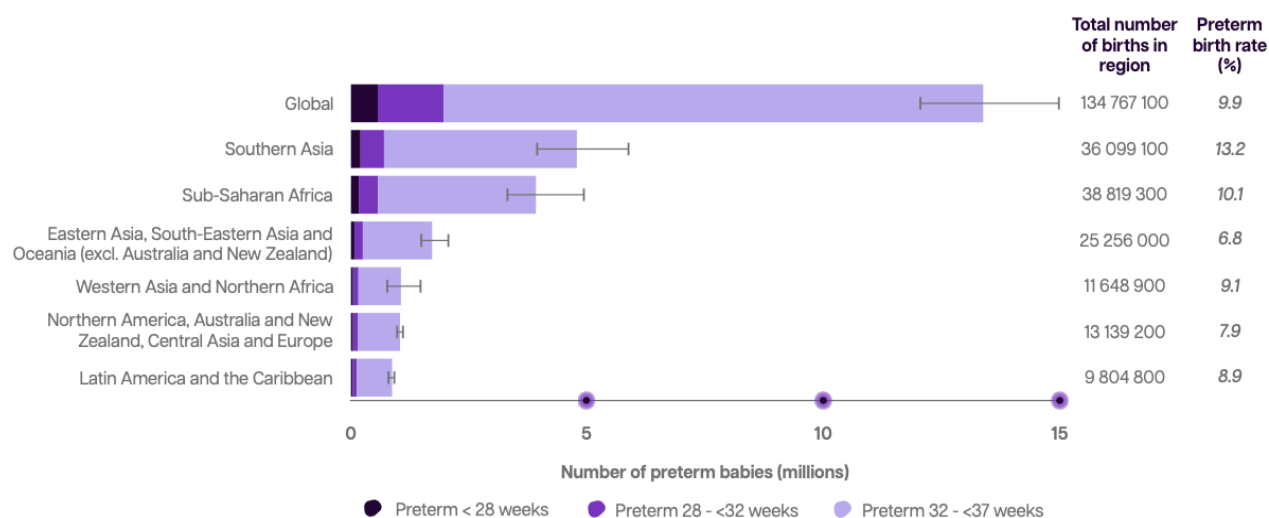


Figure 2. Regional and worldwide subgroups of preterm births in 2020

Image taken from World Health Organization, Born too soon: decade of action on preterm birth ^(6, 7).

Preterm birth ranks as the fourth major contributor to global human capital loss, surpassing chronic obstructive airways disease, diabetes, and stroke ⁽¹¹⁾. It leads to a greater burden than neonatal encephalopathy, infections, and congenital conditions, being a primary cause of disability-adjusted life years (DALYs) worldwide ⁽¹¹⁾. Prematurity stands as the leading cause of perinatal morbidity and mortality among newborns, contributing to the second leading cause of death among children under 5 years old, after pneumonia ^(1, 12).

The neonatal period, encompassing the first 28 days of life, is critical, with infants facing the highest risk of death during this time ⁽¹³⁾. In 2021, the global neonatal mortality rate was 18 deaths per 1 000 live births, showing a substantial decrease from 37 deaths per 1 000 live births in 1990 ^(13, 14). Direct complications of preterm birth contribute to almost 900,000 neonatal deaths, with 85% occurring between 32- and 36-weeks GA. Despite potential survival with neonatal intervention, the burden of DALYs primarily results from early-age deaths ⁽¹¹⁾. Furthermore, it's important to note that in developed countries, human viability, defined as the GA at which there's a 50% chance of survival, is currently around 23 to 24 weeks.

In 2021, nearly a million children succumbed to direct complications of preterm birth, highlighting a concerning lack of progress in reducing child deaths due to prematurity. Although neonatal mortality rates are slowly decreasing, they remain significant, primarily influenced by GA at birth ^(9, 13, 15). Approximately, three-quarters of these deaths could be averted through current cost-effective interventions ⁽¹⁾. Mortality rates are over 10% for infants born before 28 weeks, 5 to 10% between 28 and 31 weeks and 1 to 2% at 32 to 34 weeks of gestation ^(9, 16). Also, severe neonatal morbidity and disabilities during childhood are common and vary with GA ⁽⁹⁾.

Figure 3 illustrates a comparison between the preterm birth rates and neonatal mortality rates across different regions in 2020. It shows that neonatal mortality rates

are higher in Sub-Saharan Africa and Southern Asia compared to Latin America and the Caribbean, as well as Northern America, Europe, Australia, and New Zealand, where mortality rates are lower ⁽⁶⁾.

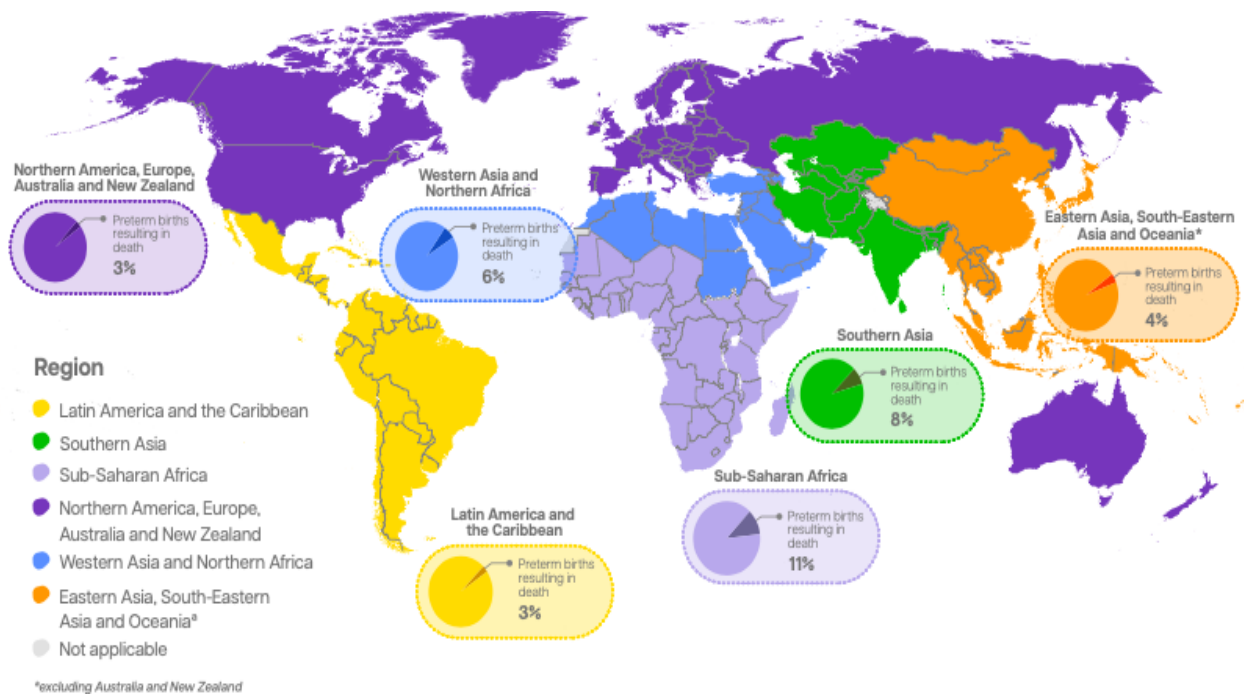


Figure 3. Regional variation in the proportion of preterm births resulting in neonatal death in 2020

Image taken from World Health Organization, *Born too soon: decade of action on preterm birth* ^(7, 8).

In Europe (2015-2019), according to the European Perinatal Health Report, neonatal mortality rates varied widely, ranging from 3.5 or more per 1000 live births in Belgium, Poland, Hungary, and Croatia to under 2.0 per 1000 in Iceland, Estonia, Sweden, and Norway ^(17, 18). The European Perinatal Health Report and WHO Data, indicated a 7,8% preterm birth prevalence rate in Portugal in 2020, remaining as one of the highest rate of prematurity on the European continent ^(17, 19). This rate has remained relatively stable over the years and, according to the latest data from the National Institute of Statistics, the preterm birth prevalence in 2017 was 8.1% ⁽¹⁹⁾. Otherwise, following National Statistics Institute and UNICEF Data, Portugal had 1,6% neonatal mortality in 2023, reflecting a substantial decrease compared with previous years, as in 1960 neonatal mortality was near 28% ⁽¹⁸⁻²⁰⁾.

1.1.2 Etiology of preterm birth

The etiology of premature birth is frequently uncertain, although specific factors can be identified. The causes of preterm birth are multifactorial and vary by gestational age, geographic and ethnic contexts ⁽²¹⁾. On the one hand, certain risk factors are related to past and present pregnancies such as multiple miscarriages or abortions, in vitro fertilization, pregnancy with twins or more, and a gap of less than six months between pregnancies ^(1, 3). Conversely, several health issues can also increase the risk of premature birth, including uterine, cervical, or placental abnormalities, infections, and ongoing health concerns such as hypertension and diabetes. Lifestyle choices also contribute to the risk of preterm pregnancies, including smoking, drugs, alcohol consumption during pregnancy, being underweight or overweight before pregnancy and being pregnant before 17 or after 37 years ^(1, 3, 12, 22-24).

As previously indicated, the prevalence of preterm births and associated mortality is predominantly concentrated in Southern Asia and sub-Saharan Africa, underscoring the global nature of the issue ⁽¹⁾. Low-income countries exhibit elevated rates of preterm births, which are influenced by a multitude of factors. These include limited access to quality healthcare services, inadequate nutrition, suboptimal maternal health conditions, heightened prevalence of infections, and insufficient awareness about prenatal care. Moreover, socioeconomic inequalities, encompassing poverty, educational deficiencies as well as and inadequate support networks, contribute to heightened stress levels among pregnant women, which in turn influence birth outcomes ^(6, 25).

1.1.3 Consequences of preterm birth

In general, there is a direct relationship between gestational age and survival rate ⁽²⁶⁾. This survival rate tends to increase as gestational ages advances, reflecting the development and maturity of organs and systems ⁽²⁶⁾. Although not all premature babies experience health complications, premature birth can lead to both short and long-term medical issues, depending on gestational age and birth weight ⁽³⁾. During pregnancy, the developing fetus undergoes a rapid growth and development, especially in the final months and weeks, as the brain, lungs, and liver require these last weeks of gestation for complete development ⁽²⁷⁾. Very and extremely preterm infants face significant health challenges and may require immediate and ongoing medical care.

A summary of the data is presented in Table 2 ^(3, 28, 29) on the immediate and long-term complications resulting from preterm birth. This reveals that one of the most significant short-term consequences is an elevated risk of readmission to the hospital and mortality following discharge. These outcomes are frequently attributed to respiratory distress and underdeveloped brain function, which can result in severe breathing issues and potential brain injury. Conversely, long-term effects include lifelong disabilities such as cerebral palsy, cognitive impairment, and sensory deficits, as well as behavioural, learning, and developmental difficulties, elevating the susceptibility to chronic diseases in adulthood ⁽²⁹⁾.

Table 2. Short and long-term complications in consequence of a preterm birth

Short-term Complications	
Breathing	– Respiratory distress syndrome (RDS) and apnoea due to undeveloped lungs. – Potential occurrence of bronchopulmonary dysplasia (BPD).
Heart	– Patent ductus arteriosus (PDA) can lead to heart failure without intervention.

	– Low blood pressure (treatments such as fluids, medications, and blood transfusions).
Brain	– Increase risk of intraventricular haemorrhage.
Temperature Control	– Lack of stored body fat and struggle to generate enough heat, leading to rapid heat loss. – Hypothermia is a dangerous condition that can cause breathing issues and low blood sugar levels.
Digestive system	– Undeveloped digestive system, increasing the risk of issues like necrotizing enterocolitis (NEC).
Blood pressures	– Anaemia resulting from a greater drop in red blood cell count during the first month. – Newborn jaundice is characterized by yellowish skin and eyes due to elevated bilirubin levels.
Metabolism	– Metabolism issues affecting the conversion of food into macronutrients.
Immune system	– Underdeveloped immune systems, increasing vulnerability and illnesses. – Infection can quickly spread to the bloodstream facing a risk of sepsis.
Long-term Complications	
Cerebral palsy	– Group of disorders affecting movement, muscle tone or posture. – Linked to factors like infection, poor blood flow or brain injury during infancy.
Trouble learning	– Experience delays in reaching developmental milestones;
Vision	– Retinopathy of prematurity causes abnormal blood vessel growth in the retina. – Vision impairment or blindness without treatment.
Hearing	– Higher risk of hearing loss.
Behaviour and mental health	– Premature birth may contribute to mental health troubles and developmental delays.

Necrotizing enterocolitis (NEC) is a severe gastrointestinal condition that is prevalent among preterm births. It affects 5-12% of very low birth weight infants and poses a significant risk of mortality in this demographic ⁽³⁰⁻³²⁾. Initial symptoms of NEC,

such as feeding intolerance, can rapidly progress to more serious complications including pneumatosis intestinalis and portal venous gas ⁽³³⁾. Surgical intervention is often required, with mortality rates ranging from 20-30%. NEC substantially extends the duration of hospitalization, with non-surgical cases requiring an additional 20 days in the neonatal intensive care unit and surgical cases necessitating an extra 60 days ^(33, 34).

Bronchopulmonary dysplasia (BPD) is a prevalent respiratory complication in preterm infants, particularly those born before 32 weeks of gestation ^(35, 36). It arises from the immaturity of the lungs and exposure to mechanical ventilation and oxygen therapy, affecting approximately 40-50% of infants weighing less than 1,500 grams ^(37, 38). Symptoms include respiratory distress and reliance on supplemental oxygen, which frequently necessitates prolonged hospitalization ^(38, 39). BPD highlights the need for strategies to prevent preterm birth and enhance neonatal care ⁽⁴⁰⁾.

1.2. Nutritional Support for Preterm

Nutritional support for preterm infants represents a crucial aspect of their care, aiming to replicate the intrauterine environment and promote optimal extrauterine growth and development ⁽⁴¹⁾. The objective of nutrition for the preterm infant is to provide nutrients that match the growth trajectory and body composition of a fetus of the same gestational age who remains in the womb until the full-term correct age ^(41, 42). As a result, the lack of adequate nutrition for preterm infants can have considerable ramifications. This not only results in growth failure but also increases the risk of morbidity and compromises neurodevelopmental outcomes ^(43, 44). Despite the advancements in neonatal care and efforts to improve nutrition protocols, many preterm infants continue to experience difficulties in achieving adequate growth rates

and often remain growth-restricted in comparison to their full-term counterparts ^(41, 45). However, providing optimal nutrition to preterm infants presents challenges, due to their physiologically unstable nature, characterized by fluctuating oxygen levels, glucose concentration, and blood pressure ⁽⁴⁶⁾. Such infants frequently require medical interventions, including the administration of catecholamines and hydrocortisone, which can further disrupt their metabolic and gastrointestinal function ^(41, 47).

Prioritizing early nutrition delivery is of paramount importance, as studies have demonstrated its potential impact on clinical outcomes, including neurodevelopment, the prevention of BPD, and the reduction of the risk of retinopathy of prematurity (ROP) ⁽⁴⁸⁻⁵⁰⁾. The administration of enhanced nutrition to extremely premature infants, both intravenously and enterally, immediately after birth has been demonstrated to promote favourable energy and protein balance, thus enhancing both long-term and short-term neurodevelopmental outcomes ^(41, 51-53). Preterm infants frequently face uncertain futures, characterized by challenges such as inadequate growth, and compromised neurodevelopmental outcomes. Therefore, optimizing their nutrition is crucial for their short- and long-term health and well-being ^(50, 54, 55). Due to its multiple benefits, the human milk is the preferred source of nutrition for preterm infants ⁽⁵⁰⁾.

Nutritional support for very premature babies, typically involves three phases:

1. Initial intensive parenteral nutrition (PN), gradually transitioning to enteral nutrition (EN) over the first few weeks after birth;
2. Introduction of fortified human milk or specialized formula once infants can tolerate full enteral feeding;
3. Managing exclusive breastfeeding or term formula after discharge ^(56, 57).

1.2.1. Parenteral and Enteral Nutrition Support

To date, research has demonstrated that early and aggressive nutritional support can enhance the growth and development of preterm infants, with positive short- and long-term outcomes for preterm infants ^(58, 59). This encompasses both parenteral and enteral nutrition, which should be initiated as soon as possible following birth. In cases where EN is not feasible due to respiratory issues or gastrointestinal immaturity, PN may be necessary initially. Nevertheless, EN is the optimal choice whenever feasible, as it facilitates intestinal function, immune development, and overall growth ⁽⁶⁰⁾. Tube feeding, either orogastric or nasogastric, is a common method of delivering feeds to preterm infants, ensuring that they receive the nutrients needed for growth and development ⁽⁶¹⁾.

The European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) recently revised their guidelines for paediatric PN, which now include specific recommendations for preterm infants ⁽⁶²⁾. Building upon this update, the Portuguese Neonatal Society has also revised their recommendations, with a particular focus on preterm infants ^(63, 64). According to these guidelines, PN should ideally begin within the first 24 hours after birth, primarily to ensure sufficient intake of glucose, calcium, and amino acids.

Despite these interventions, EN is prioritized over total PN whenever possible, to reduce potential complications such as vascular catheterization, sepsis, and adverse effects associated with it ⁽⁶⁵⁾. It is acknowledged that the early commencement of EN facilitates endocrine adaptation, promotes the maturation of motility patterns, and potentially enhances immune function ⁽⁵⁰⁾. The clinical benefits of EN include improved feed tolerance, decreased infection risk, and earlier discharge from medical care ⁽⁶⁰⁾.

Given the challenges that premature infants' face in coordinating suckling, swallowing, and breathing, tube feeding is often necessary to ensure adequate feeding

tolerance, support optimal growth, and minimize the risk of aspiration ⁽⁶⁶⁾. Consequently, orogastric or nasogastric tube feeding utilizing continuous or intermittent bolus delivery of formula or human milk is a common practice for these infants ⁽⁶⁷⁾.

1.2.2. Human milk

Breastfeeding and human milk are the gold standards for infant feeding and nutrition ⁽⁶⁸⁾. For preterm infants, human milk is prioritized due to its numerous health and developmental benefits. Human milk, containing hundreds to thousands of bioactive molecules, is the biological standard for infant nutrition ⁽⁶⁹⁾. These molecules protect against infection and inflammation, support immune maturation, promote organ development, and facilitate healthy microbial colonization ^(68, 70). It is noteworthy that certain components, such as lactoferrin, are currently being investigated for their potential as novel therapeutic agents ^(70, 71). Human milk's composition dynamically changes from colostrum to late lactation, varying by gestational age, individual feeds, diurnal rhythms, and between mothers ^(70, 72, 73). Consequently, there is a growing trend in feeding infants with expressed human milk.

According to WHO, when a mother's own milk (MOM) is unavailable, fortified donor human milk (DHM) becomes the secondary standard choice for preterm infants, conditionally recommended over preterm formulas ^(74, 75). While preterm infants are the primary recipients recommended for DHM, a recent review found that DHM is also being utilized in other groups, including healthy term infants and term infants at risk of health complications ⁽⁷⁶⁾.

To maintain the quality and safety of DHM, human milk banks (HMB) implement hazard analysis and critical control points protocols ^(76, 77). Consequently, holder pasteurization emerges as the predominant processing method of sterilization of DM

utilized in HMB, to warrant the microbiological safety ^(78, 79). However, recent studies have indicated that, while holder pasteurization ensures microbiological safety, it also modifies the composition of DM and diminishes certain nutritional components such as immunoglobulins, lactoferrin, vitamins, lysozyme, bile salt-dependent lipase, and metabolic hormones ^(76, 80). Notwithstanding, ongoing research aims to elucidate the impact of holder pasteurization on DHM in accordance with the recommendations of the European Milk Bank Association.

Globally, DHM is produced by country-level milk banking networks that are intermediaries connecting the infants in need with the human milk donors ^(75, 81). In Portugal, there are currently two human milk banks: one established in Lisbon at Maternidade Dr. Alfredo da Costa, Centro Hospitalar Universitário de Lisboa Central, and another inaugurated in Porto in 2022 at Centro Hospitalar Universitário de São João, which is fully operational and mainly serves premature infants.

1.2.3. Formula milk

Human Milk is universally acknowledged as the optimal nutrition for healthy infants, offering a comprehensive supply of nutrients crucial for growth and development ⁽⁸²⁾. While formula milk is nutritionally adequate for infants, it lacks the numerous health benefits associated with breastfeeding for both mother and child ⁽⁸³⁾. In contrast to human milk, the composition of infant formula remains consistent over time, presenting a significant challenge in attempting to mimic the dynamic nature of breast milk ⁽⁸⁴⁾. All infant formulas must adhere to safety standards and be appropriate to meet the nutritional needs and support the growth and development. This includes serving as the sole source of nutrition during the first months of life and as the primary liquid component in a gradually diversified diet with the introduction of complementary feeding ⁽⁸²⁾.

1.3. Long-chain Polyunsaturated Fatty Acid

Long-chain polyunsaturated fatty acids (LCPUFAs) are essential fatty acids (EFAs) with 18-20 carbons or more, and ≥ 3 double bonds, classified into two main groups: omega 6 (n-6) and omega 3 (n-3) ⁽⁸⁵⁻⁸⁷⁾. The primary n-3 LCPUFAs found in food sources include alpha-linolenic acid (ALA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and docosapentaenoic acid, while n-6 LCPUFAs comprise linoleic acid (LA) and arachidonic acid (AA). Intermediate products such as di-homo-gamma-linolenic acid, gamma-linolenic acid, stearidonic acid, and eicosatetraenoic acid are also produced during LCPUFA synthesis ^(85, 87-89).

Mammals, humans included, have a lack of ability to synthesize LA and ALA from the precursor oleic acid ⁽⁸⁷⁾. Consequently, these EFAs must be obtained from dietary sources, as their conversion efficiency to LCPUFAs, as AA, DHA, and EPA are low ^(85, 90). Humans can synthesize AA, DHA, and EPA, from LA and ALA, through enzymatic processes, involving desaturation and elongation. Nevertheless, the efficacy of these conversions is constrained, suggesting that direct uptake may be a more efficacious approach to addressing the identified needs ^(85, 87, 91).

1.3.1 Biological Functions

N-6 and N-3 LCPUFAs are fundamental constituents of cell membranes, crucially regulating membrane properties such as fluidity, permeability, and enzymatic activity ⁽⁹²⁾. Dietary intake significantly influences membrane composition, with increased n-3 consumption correlating with elevated n-3 content in diverse cell types, including red blood cells, immune cells, atherosclerotic plaques, and cardiac tissue ^(92, 93). LCPUFAs, not only fulfil critical structural roles within cell membranes, impacting their fluidity and function, but also act as energy reserves and metabolic regulators ⁽⁹²⁻⁹⁴⁾. Due to

this, maintaining a balanced ratio between n-6 and n-3 is imperative for overall health and disease prevention, underscoring the crucial importance of dietary intake ^(92, 93).

1.3.2. Health Benefits

LCPUFAs, especially DHA and AA, are crucial for vision and nervous system health. DHA is incorporated into retinal and neuronal cell membranes, essential for retinal development, function, and regeneration, which is vital for visual transduction ^(32, 89). In the nervous system, AA aids energy metabolism by stimulating glucose uptake in cortical astrocytes, while both AA and DHA enhance acetylcholine release, improving synaptic plasticity and memory ⁽⁹⁵⁾. LCPUFAs also maintain cellular membrane integrity and function, influencing fluidity, stability, and signalling processes. Additionally, they serve as substrates for bioactive lipid mediators like eicosanoids and docosanoids, which regulate inflammation, oxidative stress, and vascular homeostasis ⁽⁹²⁾.

Dietary n-3 LCPUFAs, such as EPA and DHA, are linked to reduced risks of chronic diseases, including cardiovascular disease and cognitive decline. Despite the positive evidence supporting the benefits of n-3 LCPUFAs, there is no consistent global recommendation for their dietary intake to meet nutritional needs and reduce the risk of non-communicable diseases ⁽⁹²⁾.

1.3.3. Food Sources

LCPUFAs, particularly n-3 and n-6 fatty acids, are essential for human health and are derived from various dietary sources. N-3 fatty acids, like EPA and DHA, are primarily found in marine sources such as fatty fish (salmon, mackerel, sardines, herring) and fish oil supplements. N-6 fatty acids, like AA, are mainly obtained from terrestrial sources, including poultry, eggs, meats, and plant-based oils (corn, soybean,

sunflower) ^(85, 96, 97). Vegetarian sources of n-3 fatty acids include flaxseeds, chia seeds, hemp seeds, and walnuts, which provide ALA ^(85, 98).

Due to the limited endogenous synthesis of LCPUFAs from LA and ALA, it is crucial to include direct dietary sources of these fatty acids. This ensures adequate levels of AA, DHA, and EPA, supporting various physiological functions and preventing deficiencies linked to chronic diseases ⁽⁹⁹⁾. Therefore, incorporating a variety of LCPUFA-rich foods while maintaining a balanced intake of n-3 and n-6 fatty acids is essential for overall health and well-being ^(89, 92, 99).

1.3.4. LCPUFA Dietary Recommendations Intake

Dietary recommendations for LCPUFAs, particularly n-3 (DHA and EPA), vary across population groups due to specific nutritional needs and health benefits. These guidelines emphasize sufficient intake, especially during critical periods like pregnancy, lactation, and early childhood, as well as for maintaining cardiovascular and cognitive health in adults. Various global authorities have set adequate intake (AI) recommendations for different age groups, underscoring the role of LCPUFAs in reducing chronic disease risk and supporting overall health ^(89, 92). Table 3 ⁽¹⁰⁰⁻¹⁰²⁾ presents AI recommendations from two main authorities: the EFSA and the FAO/WHO.

Table 3. Adequate intake of omega-3 in all populations

European Food Safety Authority (EFSA) ^(100, 101)	
Infants (6 to 24 months)	100 mg/day DHA
Children and Adolescent (2 to 18 years)	250 mg/day DHA+EPA
Adults (≥ 18 years)	250 mg/day DHA+EPA
Pregnant and lactating women	(+) 100 - 200 mg/day DHA (in addition to the adult AI)

Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO) ⁽¹⁰²⁾

Infants (6 to 24 months)	< 15% Energy
Children and Adolescent (2 to 18 years)	11% Energy
– Children (2 to 4 years)	100 - 150 mg/day DHA
– Children (4 to 6 years)	150 - 200 mg/day DHA+EPA
– Children (6 to 10 years)	200 - 250 mg/day DHA+EPA
Adults (≥ 18 years)	250 mg/day DHA+EPA
Pregnant and lactating women	300 mg/day DHA+EPA (of which 200 mg/day DHA)

Information taken from the European Food Safety Authority and World Health Organization ^(101, 102).

1.4. Long-chain Polyunsaturated Fatty Acid: The Enteral Supplementation for Preterm

During infancy, dietary lipids provide essential energy, fat-soluble vitamins, and fatty acids such as DHA and AA, which are crucial for cell membrane structure, particularly in the central nervous system ⁽¹⁰³⁾. LCPUFAs, especially DHA, play a vital role in preventing post-birth complications such as severe retinopathy of prematurity and necrotizing enterocolitis, as well as improving neurodevelopmental outcomes ⁽¹⁰⁴⁻¹⁰⁷⁾. Although the cognitive benefits of supplementation during pregnancy and infancy have shown some inconsistency, n-3 and n-6 fatty acids remain essential for brain growth and function ^(108, 109). In the first 1,000 days of life, LCPUFAs are critical for immune regulation, blood clotting, neurotransmitter function, and brain and retina development ^(93, 110, 111).

During pregnancy, DHA and AA cross the placenta to the fetus, accumulating rapidly in fetal tissues, particularly during the third trimester, a critical period for brain development ^(59, 112). After birth, these fatty acids are provided through HM, which

contains 15-20% LCPUFAs, including both precursors and metabolites, making it a primary source of essential fatty acids for infants ⁽¹¹³⁻¹¹⁵⁾. Typically, HM is the only external source of AA and DHA for infants in the first months of life. When breastfeeding is not possible, infants require human milk substitutes that are fortified to match the nutrient content of HM ^(93, 113).

Preterm infants are particularly at risk of inadequate lipid accumulation, as fetal accretion of LCPUFAs predominantly occurs in the third trimester ^(32, 107, 116, 117). During initial hospitalization, preterm infants often require parenteral nutrition to meet their nutritional needs until they reach full term. This can be challenging and may lead to postnatal malnutrition and growth failure ^(58, 118). The brain's sensitivity to nutritional intake during specific gestational periods requires an early and increased supply of energy, protein, and lipids to support growth and neurodevelopment ^(53, 115, 119). Feeding strategies for preterm infants aim to achieve growth rates that mimic fetal development, with guidelines emphasizing growth and functional development ⁽¹²⁰⁾.

After preterm birth, infants rely on external dietary sources like LCPUFA supplementation to support development and compensate for interrupted lipid accumulation ^(87, 107, 121, 122). Adequate intake of DHA and AA is crucial for brain and retina development, yet many preterm infants face a daily DHA deficit, leading to developmental challenges ^(113, 119). Human milk and preterm formulas often fall short of meeting high DHA needs, especially during the first month, resulting in a daily deficit of about 20 mg/kg ⁽¹²³⁾. Breastfeeding is preferred, with recommendations for maternal DHA supplementation or direct addition to milk ⁽¹²³⁾. Adequate energy supply during critical developmental periods is essential, as insufficient energy can lead to mobilization of fatty acids for immediate needs, compromising cell membrane formation in the central nervous system and retina ⁽¹²⁴⁻¹²⁶⁾.

Several studies report a decline in AA and DHA content after preterm birth, indicating insufficient synthesis from the precursors LA and ALA ^(115, 127, 128). Consequently, AA and DHA are considered conditionally essential fatty acids in preterm infants, with lower levels associated with complications such as retinopathy of prematurity, septicemia, and severe bronchopulmonary dysplasia ^(115, 129). Higher doses of DHA without adequate AA can decrease serum and tissue AA levels, affecting inflammation processes in the immature immune systems of preterm infants. This underscores the clinical concern of DHA supplementation in isolation compared to combined with AA ^(32, 93). Comparative studies show that term infants fed with HM have higher visual acuity and DHA concentrations than those fed with formula. Long-term research suggests that HM-fed infants may have better intelligence outcomes than formula-fed infants, as the latter typically contain greater DHA and AA ⁽³²⁾.

1.4.1. Controversy and recommendations of LCPUFA supplementation

Standard infant formulas typically contain ALA (n-3) and LA (n-6), requiring infants to synthesize DHA and AA ⁽¹³⁰⁻¹³²⁾. High LA levels can inhibit this process, leading to lower DHA and AA levels in formula-fed infants compared to those fed with HM ^(133, 134). To address this, some formula companies now add LCPUFAs, promoting them as beneficial for development ⁽¹³⁰⁾.

The EFSA and the Commission Delegated Regulation (EU) 2016/127 have defined infant formula as the only processed foodstuff that satisfies the nutritional requirements of infants during the first months of life until appropriate complementary feeding is introduced ⁽¹³⁵⁾. This regulation mandates the addition of DHA to infant and

follow-on formulas, as recommended by EFSA ^(107, 135). However, the supplementation of AA remains optional and cannot exceed 1% of the total fat content ⁽¹³⁵⁾.

Table 4. Compositional requirements of docosahexaenoic acid (DHA) in infant formulas

Minimum	Maximum
20 mg / 100 kcal	50 mg / 100kcal

Information taken from Commission Delegated Regulation (EU) 2016/127 ⁽¹³⁵⁾

The recommendations for DHA and preterm infant nutrition are primarily concerned with stable, fully enterally fed preterm infants and are based on the recently published ESPGHAN guidelines ⁽¹¹⁵⁾. This thesis will focus on the intake of fatty acids as LCPUFAs, with the exclusion of other nutrients.

The Portuguese Neonatal Society, aligning with ESPGHAN 2022 recommendations, defined guidelines for EN in infants born preterm ^(115, 136). Concerning the lipid content of the diet, it is recommended that the total fat intake for most preterm infants be between 4.8 and 8.1 grams per kilogram of body weight per day ⁽¹¹⁵⁾. To ensure that dietary fat provides 45%-55% of the energy intake, at least 4.8 g/kg/day of fat is needed to supply 96 kcal/kg/day of non-protein calories ⁽¹¹⁵⁾. Regarding fatty acids intake, the specifics are outlined in Table 5 ^(115, 136).

Table 5. Guidelines from omega-3 and omega-6 supplementation for preterm infants

Omega - 6 Recommendations	
LA Linoleic acid	385-1540 mg/kg/day
AA Arachidonic acid	30-100 mg/kg/day (recommended ¹)

Omega - 3 Recommendations

DHA Docosapentaenoic acid	30-65 mg/kg/day (strong recommendation ¹)
EPA Eicosapentaenoic acid	< 20 mg/kg/day
ALA Alpha-linolenic acid	> 55 mg/kg/day

¹ Grade of recommendation

Information taken from The Portuguese Neonatal Society, aligning with ESPGHAN 2022 recommendations ^(115, 136).

According to ESPGHAN, and as referenced in the Portuguese Neonatal Society, the safe dose for very preterm infants is administering 160-180 mL/kg/day of mature HM, which provides an average fat intake of up to 7 g/kg/day, with an upper range of about 8.1 g/kg/day ⁽¹¹⁵⁾. Additionally, an enteral supplementation with AA (30 mg/kg/day) and DHA (over 50 mg/kg/day) seems to be effective for ensuring AI of these LCPUFAs, and matching fetal blood concentrations in utero, based on the average found in HM, are recommended ⁽¹¹⁵⁾. Specifically, a DHA intake range of 30-65 mg/kg/day, an AA:DHA ratio of 0.5 to 2, and an AA intake of up to 100 mg/kg/day appear to be safe for very preterm infants ⁽¹¹⁵⁾.

While these AI guidelines are defined to meet the nutritional needs of very preterm infants, it remains to be fully established whether they are sufficient to ensure their importance in mitigating both long-term and short-term complications in this vulnerable population, especially in those born under 32 weeks of gestational age. Further research and clinical monitoring are necessary to evaluate the comprehensive impact of these guidelines on the health outcomes of very preterm infants.

2. Objective

It is hypothesized that the supplementation of very preterm infants with long-chain polyunsaturated fatty acids may yield modestly beneficial effects on various health outcomes, including neurodevelopment, growth, and metabolic factors. Furthermore, we hypothesize that the effects of long-chain polyunsaturated acid supplementation may vary based on gestational age at birth and the duration of supplementation.

This systematic review has two objectives:

- To identify the effects of long-chain polyunsaturated fatty acid supplementation on the development and maturation of preterm newborns under 32 weeks.
- To determine whether there are doses higher than the established safety threshold that can be administered to preterm infants to improve health outcomes without causing harm.

3. Methods

To answer the aims of this thesis, a systematic review was undertaken, highlighting the increasing importance of this type of study in health care. In response to this objective, an increasing number of researchers are reading/writing systematic reviews, recognizing their significance in synthesizing existing evidence comprehensively to answer specific research questions.

The Cochrane Review is defined as a systematic review that identifies, evaluates, and synthesizes all eligible evidence to address specific primary research on a particular question. It is based on explicit and systematic methods and minimizes bias for more reliable findings. Certainly, owing to the potential for continuous updates influenced by novel studies and emerging findings, Cochrane Reviews remain dynamic and pertinent reservoirs of information providing sustained insights into the research questions at hand ⁽¹³⁷⁾.

One of the main and initial steps during the development of a systematic review is registering it on the International Prospective Register of Systematic Reviews (PROSPERO) platform. This step is crucial because PROSPERO, as an international database, ensures transparency by registering systematic reviews at the inception. It also helps prevent duplication, and reduces reporting bias, contributing to the overall quality and reliability of research outcomes. Thus, this review was pre-registered in advance on this platform (PROSPERO: CRD42023461646) ⁽¹³⁸⁾.

4. References of background and methods

1. World Health Organization. Preterm birth. 2023. Available at: <https://www.who.int/news-room/fact-sheets/detail/preterm-birth>. Accessed January 2024.
2. Quinn JA, Munoz FM, Gonik B, Frau L, Cutland C, Mallett-Moore T, et al. Preterm birth: Case definition & guidelines for data collection, analysis, and presentation of immunisation safety data. *Vaccine*. 2016; 34(49):6047-56. <https://doi.org/10.1016/j.vaccine.2016.03.045>.
3. Mayo Clinic. Premature birth. 2023. Available at: <https://www.mayoclinic.org/diseases-conditions/premature-birth/symptoms-causes/syc-20376730>. Accessed January 2024.
4. American College of Obstetricians and Gynecologists Committee Opinion No 579: Definition of term pregnancy. *Obstet Gynecol*. 2013; 122(5):1139-40. <https://doi.org/10.1097/01.aog.0000437385.88715.4a>.
5. Medically Indicated Late-Preterm and Early-Term Deliveries: ACOG Committee Opinion, Number 818. *Obstet Gynecol*. 2021; 137(2):e29-e33. <https://doi.org/10.1097/aog.0000000000004245>.
6. World Health Organization. Born too soon: decade of action on preterm birth. World Health Organization; 2023. ISBN: 978-92-4-007389-0.
7. Ohuma EO, Moller A-B, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *The Lancet*. 2023; 402(10409):1261-71. [http://dx.doi.org/10.1016/S0140-6736\(23\)00878-4](http://dx.doi.org/10.1016/S0140-6736(23)00878-4).
8. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, et al. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and

implications. *Lancet*. 2012; 379(9832):2162-72. [https://doi.org/10.1016/s0140-6736\(12\)60820-4](https://doi.org/10.1016/s0140-6736(12)60820-4).

9. Torchin H, Ancel PY, Jarreau PH, Goffinet F. [Epidemiology of preterm birth: Prevalence, recent trends, short- and long-term outcomes]. *J Gynecol Obstet Biol Reprod (Paris)*. 2015; 44(8):723-31. <https://doi.org/10.1016/j.jgyn.2015.06.010>.

10. De Costa A, Moller A-B, Blencowe H, Johansson EW, Hussain-Alkhateeb L, Ohuma EO, et al. Study protocol for WHO and UNICEF estimates of global, regional, and national preterm birth rates for 2010 to 2019. *PLoS One*. 2021; 16(10):e0258751. <https://doi.org/10.1371/journal.pone.0258751>.

11. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020; 396(10258):1204-22. [https://doi.org/10.1016/s0140-6736\(20\)30925-9](https://doi.org/10.1016/s0140-6736(20)30925-9).

12. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *The lancet*. 2008; 371(9606):75-84. [https://doi.org/10.1016/s0140-6736\(08\)60074-4](https://doi.org/10.1016/s0140-6736(08)60074-4).

13. United Nations International Children's Emergency Fund. Neonatal mortality. 2023. Available at: <https://data.unicef.org/topic/child-survival/neonatal-mortality/>. Accessed march 2024.

14. Alexander M, Alkema L. Global estimation of neonatal mortality using a Bayesian hierarchical splines regression model. *Demographic Research*. 2018; 38:335-72. <https://doi.org/10.4054/DemRes.2018.38.15>.

15. World Health Organization. Newborn Mortality. 2022. Available at: <https://www.who.int/news-room/fact-sheets/detail/levels-and-trends-in-child-mortality-report-2021>. Accessed march 2024.

16. Markestad T, Kaaresen PI, Rønnestad A, Reigstad H, Lossius K, Medbø S, et al. Early death, morbidity, and need of treatment among extremely premature infants. *Pediatrics*. 2005; 115(5):1289-98. <https://doi.org/10.1542/peds.2004-1482>.
17. Euro-Peristat. European Perinatal Health Report: Health and Care of Pregnant Women and Babies in Europe from 2015 to 2019. 2019
18. Institutos Nacionais de Estatística PORDATA. Taxas de mortalidade neonatal e taxa de mortalidade fetal tardia. 2023. Available at: <https://www.pordata.pt/europa/taxas+de+mortalidade+neonatal+e+taxa+de+mortalidade+fetal+tardia-1258>. Accessed may 2024.
19. Instituto Nacional de Estatística. Neonatal mortality rate. Available at: <http://www.ine.pt>. Accessed may 2024.
20. United Nations International Children's Emergency Fund. UNICEF Data Warehouse. Available at: https://data.unicef.org/resources/data_explorer/unicef_f/?ag=UNICEF&df=GLOBAL_DATAFLOW&ver=1.0&dq=PRT.CME_TMM0+CME_PND+CME_MRM0.&startPeriod=2018&endPeriod=2024. Accessed april 2024.
21. Simmons LE, Rubens CE, Darmstadt GL, Gravett MG. Preventing preterm birth and neonatal mortality: exploring the epidemiology, causes, and interventions. *Semin Perinatol*. 2010; 34(6):408-15. <https://doi.org/10.1053/j.semperi.2010.09.005>.
22. *the American College of Obstetricians and Gynecologists*. FAQs: Preterm Labor and Birth. april 2023. Available at: <https://www.acog.org/womens-health/faqs/preterm-labor-and-birth>. Accessed april 2024.
23. Kliegman RM ea. The Newborn. *Nelson Textbook of Pediatrics*. 2020; 21st ed
24. Iams JD. Prevention of preterm parturition. *New England Journal of Medicine*. 2014; 370(3):254-61. <https://doi.org/10.1056/nejmcp1103640>.

25. Rahman MM, Abe SK, Rahman MS, Kanda M, Narita S, Bilano V, et al. Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis. *Am J Clin Nutr.* 2016; 103(2):495-504. <https://doi.org/10.3945/ajcn.115.107896>.
26. *the American College of Obstetricians and Gynecologists*. FAQs: Extremely Preterm Birth. Available at: <https://www.acog.org/womens-health/faqs/extremely-preterm-birth>. Accessed april 2024.
27. Centers for Disease Control and Prevention. Preterm Birth. 2023. Available at: <https://www.cdc.gov/reproductivehealth/maternalinfanthealth/pretermbirth.htm>. Accessed april 2024.
28. Patel RM. Short-and long-term outcomes for extremely preterm infants. *American journal of perinatology.* 2016:318-28. <https://doi.org/10.1055%2Fs-0035-1571202>.
29. Short and Long-Term Effects of Preterm Birth. UK HealthCare Brand Strategy. Available at: <https://ukhealthcare.uky.edu/wellness-community/health-information/short-long-term-effects-preterm-birth>. Accessed april 2024.
30. Fox TP, Godavitarne C. What really causes necrotising enterocolitis? *ISRN Gastroenterol.* 2012; 2012:628317. <https://doi.org/10.5402%2F2012%2F628317>.
31. Youn YA, Kim EK, Kim SY. Necrotizing Enterocolitis among Very-Low-Birth-Weight Infants in Korea. *J Korean Med Sci.* 2015; 30 Suppl 1(Suppl 1):S75-80. <https://doi.org/10.3346%2Fjkms.2015.30.S1.S75>.
32. Alshaikh BN, Reyes Loredó A, Yusuf K, Maarouf A, Fenton TR, Momin S. Enteral long-chain polyunsaturated fatty acids and necrotizing enterocolitis: A systematic review and meta-analysis. *Am J Clin Nutr.* 2023; 117(5):918-29. <https://doi.org/10.1016/j.ajcnut.2023.01.007>.

33. Meister AL, Doheny KK, Travagli RA. Necrotizing enterocolitis: It's not all in the gut. *Exp Biol Med* (Maywood). 2020; 245(2):85-95. <https://doi.org/10.1177/1535370219891971>.
34. Neu J, Walker WA. Necrotizing enterocolitis. *N Engl J Med*. 2011; 364(3):255-64. <https://doi.org/10.1056%2FNEJMra1005408>.
35. Khetan R, Hurley M, Spencer S, Bhatt JM. Bronchopulmonary Dysplasia Within and Beyond the Neonatal Unit. *Adv Neonatal Care*. 2016; 16(1):17-25;quiz E1-2. <https://doi.org/10.1097/anc.0000000000000251>.
36. Thébaud B, Goss KN, Laughon M, Whitsett JA, Abman SH, Steinhorn RH, et al. Bronchopulmonary dysplasia. *Nat Rev Dis Primers*. 2019; 5(1):78. <https://doi.org/10.1038/s41572-019-0127-7>.
37. Jensen EA, Schmidt B. Epidemiology of bronchopulmonary dysplasia. *Birth Defects Res A Clin Mol Teratol*. 2014; 100(3):145-57. <https://doi.org/10.1002/bdra.23235>.
38. National Heart Lung, and Blood Institute. Bronchopulmonary Dysplasia (BPD). 2022. Available at: <https://www.nhlbi.nih.gov/health/bronchopulmonary-dysplasia>. Accessed april 2024.
39. American Lung Association. Bronchopulmonary Dysplasia Symptoms and Diagnosis. 2022. Available at: <https://www.lung.org/lung-health-diseases/lung-disease-lookup/bronchopulmonary-dysplasia/symptoms-diagnosis>. Accessed april 2024.
40. Aschner JL, Bancalari EH, McEvoy CT. Can We Prevent Bronchopulmonary Dysplasia? *J Pediatr*. 2017; 189:26-30. <https://doi.org/10.1016%2Fj.jpeds.2017.08.005>.
41. Hay WW, Jr. Nutritional Support Strategies for the Preterm Infant in the Neonatal Intensive Care Unit. *Pediatr Gastroenterol Hepatol Nutr*. 2018; 21(4):234-47. <https://doi.org/10.5223%2Fpghn.2018.21.4.234>.

42. Kleinman RE, Greer FR, Pediatrics AAP. Pediatric nutrition. American Academy of Pediatrics Elk Grove Village, IL; 2014.
43. Bouyssi-Kobar M, du Plessis AJ, McCarter R, Brossard-Racine M, Murnick J, Tinkleman L, et al. Third trimester brain growth in preterm infants compared with in utero healthy fetuses. *Pediatrics*. 2016; 138(5). <https://doi.org/10.1542/peds.2016-1640>.
44. Ehrenkranz RA, Dusick AM, Vohr BR, Wright LL, Wrage LA, Poole WK, et al. Growth in the neonatal intensive care unit influences neurodevelopmental and growth outcomes of extremely low birth weight infants. *Pediatrics*. 2006; 117(4):1253-61. <https://doi.org/10.1542/peds.2005-1368>.
45. Horbar JD, Ehrenkranz RA, Badger GJ, Edwards EM, Morrow KA, Soll RF, et al. Weight growth velocity and postnatal growth failure in infants 501 to 1500 grams: 2000-2013. *Pediatrics*. 2015; 136(1):e84-e92. <https://doi.org/10.1542/peds.2015-0129>.
46. Hay WW. Aggressive nutrition of the preterm infant. *Current pediatrics reports*. 2013; 1:229-39. <https://doi.org/10.1007/s40124-013-0026-4>.
47. YiehLin C, Bloomfield F, O'Sullivan J. Factors affecting gastrointestinal microbiome development in neonates. 2018. <https://doi.org/10.3390/nu10030274>.
48. Alanazi M, Altawili MA, Khayyal Al, Alahmari AS, Alhakami AA, Alshehri AMA. Impact of Early Nutrition Interventions on the Growth and Development of Preterm Infants: A Narrative Review. *Cureus*. 2024; 16(2):e54888. <https://doi.org/10.7759/cureus.54888>.
49. Schneider N, Garcia-Rodenas CL. Early Nutritional Interventions for Brain and Cognitive Development in Preterm Infants: A Review of the Literature. *Nutrients*. 2017; 9(3). <https://doi.org/10.3390/nu9030187>.

50. Thoene M, Anderson-Berry A. Early Enteral Feeding in Preterm Infants: A Narrative Review of the Nutritional, Metabolic, and Developmental Benefits. *Nutrients*. 2021; 13(7). <https://doi.org/10.3390%2Fnu13072289>.
51. Dinerstein A, Nieto RM, Solana CL, Perez GP, Otheguy LE, Larguia AM. Early and aggressive nutritional strategy (parenteral and enteral) decreases postnatal growth failure in very low birth weight infants. *J Perinatol*. 2006; 26(7):436-42. <https://doi.org/10.1038/sj.jp.7211539>.
52. Likhar A, Patil MS. Importance of Maternal Nutrition in the First 1,000 Days of Life and Its Effects on Child Development: A Narrative Review. *Cureus*. 2022; 14(10):e30083. <https://doi.org/10.7759%2Fcureus.30083>.
53. Silveira RC, Corso AL, Procianoy RS. The Influence of Early Nutrition on Neurodevelopmental Outcomes in Preterm Infants. *Nutrients*. 2023; 15(21). <https://doi.org/10.3390/nu15214644>.
54. Su B-H. Optimizing Nutrition in Preterm Infants. *Pediatrics & Neonatology*. 2014; 55(1):5-13. <https://doi.org/10.1016/j.pedneo.2013.07.003>.
55. Hee Chung E, Chou J, Brown KA. Neurodevelopmental outcomes of preterm infants: a recent literature review. *Transl Pediatr*. 2020; 9(Suppl 1):S3-s8. <https://doi.org/10.21037/tp.2019.09.10>.
56. Su BH. Optimizing nutrition in preterm infants. *Pediatr Neonatol*. 2014; 55(1):5-13. <https://doi.org/10.1016/j.pedneo.2013.07.003>.
57. Cardoso MM. Crescimento e composição corporal de crianças nascidas muito pré-termo: comparação entre duas coortes alimentadas com leite humano, respetivamente com fortificação alvo e padrão. 2024
58. Hay WW, Jr. Aggressive Nutrition of the Preterm Infant. *Curr Pediatr Rep*. 2013; 1(4). <https://doi.org/10.1007%2Fs40124-013-0026-4>.

59. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2010; 50(1):85-91. <https://doi.org/10.1097/mpg.0b013e3181adaee0>.
60. Bozzetti V, Tagliabue PE. Enteral nutrition for preterm infants: by bolus or continuous? An update. *Pediatr Med Chir.* 2017; 39(2):159. <https://doi.org/10.4081/pmc.2017.159>.
61. Sadrudin Premji S, Chessell L, Stewart F. Continuous nasogastric milk feeding versus intermittent bolus milk feeding for preterm infants less than 1500 grams. *Cochrane Database Syst Rev.* 2021; 6(6):Cd001819. <https://doi.org/10.1002/14651858.cd001819.pub3>.
62. Mihatsch W, Shamir R, van Goudoever JB, Fewtrell M, Lapillonne A, Lohner S, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Guideline development process for the updated guidelines. *Clin Nutr.* 2018; 37(6 Pt B):2306-08. <https://doi.org/10.1016/j.clnu.2018.06.943>.
63. Pereira-da-Silva L, Pissarra S, Alexandrino AM, Malheiro L, Macedo I, Cardoso M, et al. Guidelines for neonatal parenteral nutrition: 2019 update by the Portuguese Neonatal Society. Part II. Micronutrients, ready-to-use solutions and particular conditions. *Energy (kcal/kg/day).* 2019; 45(55):90-120. <https://doi.org/10.25754/pjp.2019.16027>.
64. Pereira-da-Silva L, Pissarra S, Alexandrino AM, Malheiro L, Macedo I, Cardoso M, et al. Guidelines for Neonatal Parenteral Nutrition: 2019 Update by the Portuguese Neonatal Society. Part I. General Aspects, Energy, and Macronutrients. 2019. <https://doi.org/10.25754/pjp.2019.15981>.

65. Khan AA, Khan A, Khan MA, Kumar D, Naquvi KJ. Total parenteral nutrition: A boon to non-functional gastrointestinal patients. *Intelligent Pharmacy*. 2024; 2(2):263-69. <http://dx.doi.org/10.1016/j.ipha.2023.10.007>.
66. Kamity R, Kapavarapu PK, Chandel A. Feeding Problems and Long-Term Outcomes in Preterm Infants-A Systematic Approach to Evaluation and Management. *Children (Basel)*. 2021; 8(12). <https://doi.org/10.3390/children8121158>.
67. Rogers SP, Hicks PD, Hamzo M, Veit LE, Abrams SA. Continuous feedings of fortified human milk lead to nutrient losses of fat, calcium and phosphorous. *Nutrients*. 2010; 2(3):230-40. <https://doi.org/10.3390/nu2030240>.
68. BREASTFEEDING SO, Eidelman AI, Schanler RJ, Johnston M, Landers S, Noble L, et al. Breastfeeding and the Use of Human Milk. *Pediatrics*. 2012; 129(3):e827-e41. <https://doi.org/10.1542/peds.2011-3552>.
69. Cerasani J, Ceroni F, De Cosmi V, Mazzocchi A, Morniroli D, Roggero P, et al. Human Milk Feeding and Preterm Infants' Growth and Body Composition: A Literature Review. *Nutrients*. 2020; 12(4). <https://doi.org/10.3390/nu12041155>.
70. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. *Pediatr Clin North Am*. 2013; 60(1):49-74. <https://doi.org/10.1016%2Fj.pcl.2012.10.002>.
71. Nolan LS, Rimer JM, Good M. The Role of Human Milk Oligosaccharides and Probiotics on the Neonatal Microbiome and Risk of Necrotizing Enterocolitis: A Narrative Review. *Nutrients*. 2020; 12(10). <https://doi.org/10.3390/nu12103052>.
72. Meredith-Dennis L, Xu G, Goonatilleke E, Lebrilla CB, Underwood MA, Smilowitz JT. Composition and Variation of Macronutrients, Immune Proteins, and Human Milk Oligosaccharides in Human Milk From Nonprofit and Commercial Milk Banks. *J Hum Lact*. 2018; 34(1):120-29. <https://doi.org/10.1177/0890334417710635>.

73. Gila-Diaz A, Arribas SM, Algara A, Martín-Cabrejas MA, López de Pablo Á L, Sáenz de Pipaón M, et al. A Review of Bioactive Factors in Human Breastmilk: A Focus on Prematurity. *Nutrients*. 2019; 11(6). <https://doi.org/10.3390/nu11061307>.
74. Parker LA, Koernere R, Fordham K, Bubshait H, Eugene A, Gefre A, et al. Mother's Own Milk Versus Donor Human Milk: What's the Difference? *Crit Care Nurs Clin North Am*. 2024; 36(1):119-33. <https://doi.org/10.1016/j.cnc.2023.09.002>.
75. World Health Organization. Donor Human Milk for Low-Birth-Weight Infants. 2019. Available at: http://www.who.int/elena/titles/donormilk_infants/en/. Accessed at april 2024.
76. Gutierrez Dos Santos B, Perrin MT. What is known about human milk bank donors around the world: a systematic scoping review. *Public Health Nutr*. 2022; 25(2):312-22. <https://doi.org/10.1017/s1368980021003979>.
77. PATH. Strengthening human milk banking: A global implementation framework. Bill and Melinda Gates Foundation Grand Challenges Initiative Seattle, WA; 2013.
78. Carneiro L, Marousez L, Van Hul M, Tran LC, De Lamballerie M, Ley D, et al. The Sterilization of Human Milk by Holder Pasteurization or by High Hydrostatic Pressure Processing Leads to Differential Intestinal Effects in Mice. *Nutrients*. 2023; 15(18). <https://doi.org/10.3390/nu15184043>.
79. Weaver G, Bertino E, Gebauer C, Grovslie A, Mileusnic-Milenovic R, Arslanoglu S, et al. Recommendations for the establishment and operation of human milk banks in Europe: a consensus statement from the European Milk Bank Association (EMBA). *Frontiers in pediatrics*. 2019; 7:53. <https://doi.org/10.3389/fped.2019.00053>.
80. Peila C, Moro GE, Bertino E, Cavallarin L, Giribaldi M, Giuliani F, et al. The Effect of Holder Pasteurization on Nutrients and Biologically-Active Components in Donor Human Milk: A Review. *Nutrients*. 2016; 8(8). <https://doi.org/10.3390/nu8080477>

81. European Milk Banking Association. 2021. Available at: <https://europeanmilkbanking.com/>. Accessed april 2024.
82. Agostoni C, Berni Canani R, Fairweather Tait S, Heinonen M, Korhonen H, La Vieille S, et al. Scientific opinion on the essential composition of infant and follow-on formulae. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). EFSA JOURNAL. 2014; 12(7):1-106. <https://doi.org/10.2903/j.efsa.2014.3760>.
83. National Health Service. Types of formula. 2023. Available at: <https://www.nhs.uk/conditions/baby/breastfeeding-and-bottle-feeding/bottle-feeding/types-of-formula/>. Accessed may 2024.
84. Institute of Medicine (US) Committee on the Evaluation of the Addition of Ingredients New to Infant Formula. Infant formula: evaluating the safety of new ingredients. National Academies Press; 2004. <https://doi.org/10.17226/10935>
85. Abedi E, Sahari MA. Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. Food Sci Nutr. 2014; 2(5):443-63. <https://doi.org/10.1002%2Ffsn3.121>.
86. Falsini B, Sieving P, Bush R. Neuroprotection Benedetto Falsini, Ronald A. Bush, Paul A. Sieving. In: Retina Vth Edition. Ryan S; 2013. p. 716-33.
87. Heath RJ, Klevebro S, Wood TR. Maternal and Neonatal Polyunsaturated Fatty Acid Intake and Risk of Neurodevelopmental Impairment in Premature Infants. Int J Mol Sci. 2022; 23(2). <https://doi.org/10.3390/ijms23020700>.
88. Simopoulos AP. The omega-6/omega-3 fatty acid ratio: health implications. Oléagineux, Corps gras, Lipides. 2010; 17(5):267-75. <http://dx.doi.org/10.1684/ocl.2010.0325>.
89. Van Dael P. Role of n-3 long-chain polyunsaturated fatty acids in human nutrition and health: review of recent studies and recommendations. Nutr Res Pract. 2021; 15(2):137-59. <https://doi.org/10.4162%2Fnrp.2021.15.2.137>.

90. Horrocks LA, Yeo YK. Health benefits of docosahexaenoic acid (DHA). *Pharmacol Res.* 1999; 40(3):211-25. <https://doi.org/10.1006/phrs.1999.0495>.
91. Innis SM. Dietary (n-3) fatty acids and brain development. *J Nutr.* 2007; 137(4):855-9. <https://doi.org/10.1093/jn/137.4.855>.
92. Higdon J. Essential fatty acids. Linus Pauling Institute at Oregon State University. 2003. Available at: <https://lpi.oregonstate.edu/mic/other-nutrients/essential-fatty-acids>. Accessed may 2024.
93. Salas Lorenzo I, Chisaguano Tonato AM, de la Garza Puentes A, Nieto A, Herrmann F, Dieguez E, et al. The Effect of an Infant Formula Supplemented with AA and DHA on Fatty Acid Levels of Infants with Different FADS Genotypes: The COGNIS Study. *Nutrients.* 2019; 11(3). <https://doi.org/10.3390%2Fnu11030602>.
94. Torres M, Parets S, Fernández-Díaz J, Beteta-Göbel R, Rodríguez-Lorca R, Román R, et al. Lipids in Pathophysiology and Development of the Membrane Lipid Therapy: New Bioactive Lipids. *Membranes* (Basel). 2021; 11(12). <https://doi.org/10.3390%2Fmembranes11120919>.
95. Brenna JT, Diau GY. The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids.* 2007; 77(5-6):247-50. <https://doi.org/10.1016/j.plefa.2007.10.016>.
96. Howe P, Meyer B, Record S, Baghurst K. Dietary intake of long-chain omega-3 polyunsaturated fatty acids: contribution of meat sources. *Nutrition.* 2006; 22(1):47-53. <https://doi.org/10.1016/j.nut.2005.05.009>.
97. Williams CM, Burdge G. Long-chain n-3 PUFA: plant v. marine sources. *Proc Nutr Soc.* 2006; 65(1):42-50. <https://doi.org/10.1079/pns2005473>.

98. Sanders TA. Essential fatty acid requirements of vegetarians in pregnancy, lactation, and infancy. *Am J Clin Nutr.* 1999; 70(3 Suppl):555s-59s. <https://doi.org/10.1093/ajcn/70.3.555s>.
99. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. *Biochem Soc Trans.* 2017; 45(5):1105-15. <https://doi.org/10.1042/bst20160474>.
100. The European Food Safety Authority. Dietary Reference Values for the EU. may 13, 2024. Disponível em: <https://multimedia.efsa.europa.eu/drvs/index.htm>.
101. The European Food Safety Authority Panel on Dietetic Products N, Allergies. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *Efsa Journal.* 2010; 8(3):1461.
102. Joint F. Fats and fatty acids in human nutrition. Report of an expert consultation, 10-14 November 2008, Geneva. 2010
103. Uauy R, Castillo C. Lipid Requirements of Infants: Implications for Nutrient Composition of Fortified Complementary Foods. *The Journal of Nutrition.* 2003; 133(9):2962S-72S. <https://doi.org/10.1093/jn/133.9.2962s>.
104. Guillot M, Synnes A, Pronovost E, Qureshi M, Daboval T, Caouette G, et al. Maternal High-Dose DHA Supplementation and Neurodevelopment at 18-22 Months of Preterm Children. *Pediatrics.* 2022; 150(1). <https://doi.org/10.1542/peds.2021-055819>.
105. Hellström A, Pivodic A, Gränse L, Lundgren P, Sjöbom U, Nilsson AK, et al. Association of Docosahexaenoic Acid and Arachidonic Acid Serum Levels With Retinopathy of Prematurity in Preterm Infants. *JAMA Netw Open.* 2021; 4(10):e2128771. <https://doi.org/10.1001/jamanetworkopen.2021.28771>.
106. Singh P, Ochoa-Allemant P, Brown J, Perides G, Freedman SD, Martin CR. Effect of polyunsaturated fatty acids on postnatal ileum development using the fat-1

transgenic mouse model. *Pediatr Res.* 2019; 85(4):556-65.
<https://doi.org/10.1038/s41390-019-0284-0>.

107. Alshaikh BN, Loredó AR, Yusuf K, Maarouf A, Fenton TR, Momin S. Enteral long-chain polyunsaturated fatty acids and necrotizing enterocolitis: A systematic review and meta-analysis. *The American Journal of Clinical Nutrition.* 2023; 117(5):918-29.
<https://doi.org/10.1016/j.ajcnut.2023.01.007>.

108. Lapillonne A, Moltu SJ. Long-Chain Polyunsaturated Fatty Acids and Clinical Outcomes of Preterm Infants. *Ann Nutr Metab.* 2016; 69 Suppl 1:35-44.
<https://doi.org/10.1159/000448265>.

109. Dighriri IM, Alsubaie AM, Hakami FM, Hamithi DM, Alshekh MM, Khobrani FA, et al. Effects of Omega-3 Polyunsaturated Fatty Acids on Brain Functions: A Systematic Review. *Cureus.* 2022; 14(10):e30091. <https://doi.org/10.7759/cureus.30091>.

110. Shahabi B, Hernández-Martínez C, Voltas N, Canals J, Arija V. The Maternal Omega-3 Long-Chain Polyunsaturated Fatty Acid Concentration in Early Pregnancy and Infant Neurodevelopment: The ECLIPSES Study. *Nutrients.* 2024; 16(5).
<https://doi.org/10.3390/nu16050687>

111. Cusick SE, Georgieff MK. The Role of Nutrition in Brain Development: The Golden Opportunity of the "First 1000 Days". *J Pediatr.* 2016; 175:16-21.
<https://doi.org/10.1016%2Fj.jpeds.2016.05.013>.

112. Georgieff MK, Innis SM. Controversial nutrients that potentially affect preterm neurodevelopment: essential fatty acids and iron. *Pediatr Res.* 2005; 57(5 Pt 2):99r-103r. <https://doi.org/10.1203/01.pdr.0000160542.69840.0f>.

113. Moon K, Rao SC, Schulzke SM, Patole SK, Simmer K. Longchain polyunsaturated fatty acid supplementation in preterm infants. *Cochrane Database Syst Rev.* 2016; 12(12):Cd000375. <https://doi.org/10.1002/14651858.cd000375.pub5>.

114. Grote V, Verduci E, Scaglioni S, Vecchi F, Contarini G, Giovannini M, et al. Breast milk composition and infant nutrient intakes during the first 12 months of life. *European journal of clinical nutrition*. 2016; 70(2):250-56. <https://doi.org/10.1038/ejcn.2015.162>.
115. Embleton ND, Moltu SJ, Lapillonne A, Van Den Akker CH, Carnielli V, Fusch C, et al. Enteral nutrition in preterm infants (2022): a position paper from the ESPGHAN committee on nutrition and invited experts. *Journal of pediatric gastroenterology and nutrition*. 2023; 76(2):248-68. <https://doi.org/10.1097/mpg.0000000000003642>.
116. Uauy R, Mena P, Wegher B, Nieto S, Salem N, Jr. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. *Pediatr Res*. 2000; 47(1):127-35. <https://doi.org/10.1203/00006450-200001000-00022>.
117. Kuipers RS, Luxwolda MF, Offringa PJ, Martini IA, Boersma ER, Dijck-Brouwer DJ, et al. Gestational age dependent content, composition and intrauterine accretion rates of fatty acids in fetal white adipose tissue. *Prostaglandins, leukotrienes and essential fatty acids*. 2012; 86(1-2):39-49. <https://doi.org/10.3390/nu13062061>.
118. Wiechers C, Bernhard W, Goelz R, Poets CF, Franz AR. Optimizing Early Neonatal Nutrition and Dietary Pattern in Premature Infants. *Int J Environ Res Public Health*. 2021; 18(14). <https://doi.org/10.3390/ijerph18147544>.
119. Crawford M. Placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of preterm infants. *The American journal of clinical nutrition*. 2000; 71(1):275S-84S. <https://doi.org/10.1093/ajcn/71.1.275s>.
120. Ziegler EE. Nutrient requirements of premature infants. *Nestle Nutr Workshop Ser Pediatr Program*. 2007; 59:161-72; discussion 72-6. <https://doi.org/10.1159/000098534>.

121. Clandinin M, Chappell J, Leong S, Heim T, Swyer P, Chance G. Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early human development*. 1980; 4(2):131-38. [https://doi.org/10.1016/0378-3782\(80\)90016-x](https://doi.org/10.1016/0378-3782(80)90016-x).
122. Fleith M, Clandinin M. Dietary PUFA for preterm and term infants: review of clinical studies. *Critical reviews in food science and nutrition*. 2005; 45(3):205-29. <https://doi.org/10.1080/10408690590956378>.
123. Lapillonne A, Jensen CL. Reevaluation of the DHA requirement for the premature infant. *Prostaglandins Leukot Essent Fatty Acids*. 2009; 81(2-3):143-50. <https://doi.org/10.1016/j.plefa.2009.05.014>.
124. Herrera E. Implications of Dietary Fatty Acids During Pregnancy on Placental, Fetal and Postnatal Development—A Review. *Placenta*. 2002; 23:S9-S19. <https://doi.org/10.1053/plac.2002.0771>.
125. Innis SM. Dietary (n-3) Fatty Acids and Brain Development¹². *The Journal of Nutrition*. 2007; 137(4):855-59. <https://doi.org/10.1093/jn/137.4.855>.
126. Lapillonne A, Jensen CL. Reevaluation of the DHA requirement for the premature infant. *Prostaglandins, Leukotrienes and Essential Fatty Acids*. 2009; 81(2):143-50. <https://doi.org/10.1016/j.plefa.2009.05.014>.
127. Bernard JY, De Agostini M, Forhan A, de Lauzon-Guillain B. The Dietary n6: n3 Fatty Acid Ratio during Pregnancy Is Inversely Associated with Child Neurodevelopment in the EDEN Mother-Child. <https://doi.org/10.3945/jn.113.178640>.
128. Kitamura T, Kitamura Y, Hamano H, Shoji H, Shimizu T, Shimizu T. The ratio of docosahexaenoic acid and arachidonic acid in infant formula influences the fatty acid composition of the erythrocyte membrane in low-birth-weight infants. *Annals of Nutrition and Metabolism*. 2016; 68(2):103-12. <https://doi.org/10.1159/000443024>.
129. Koletzko B, Lapillonne A. Lipid requirements of preterm infants. *Nutritional Care of Preterm Infants*. 2021; 122:89-102. <https://doi.org/10.1159/000514749>.

130. Jasani B, Simmer K, Patole SK, Rao SC. Long chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev*. 2017; 3(3):Cd000376. <https://doi.org/10.1002/14651858.cd000376.pub4>.
131. Salem Jr N, Wegher B, Mena P, Uauy R. Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proceedings of the National Academy of Sciences*. 1996; 93(1):49-54. <https://doi.org/10.1073/pnas.93.1.49>.
132. Gibson RA. Long-chain polyunsaturated fatty acid (LCPUFA) requirement for brain development: A personal view. *OCL*. 2016; 23(1):D115. <https://doi.org/10.1051/ocl/2015039>.
133. Fleith M, Clandinin MT. Dietary PUFA for preterm and term infants: review of clinical studies. *Crit Rev Food Sci Nutr*. 2005; 45(3):205-29. <https://doi.org/10.1080/10408690590956378>.
134. Sanders TA, Naismith DJ. A comparison of the influence of breast-feeding and bottle-feeding on the fatty acid composition of the erythrocytes. *Br J Nutr*. 1979; 41(3):619-23. <https://doi.org/10.1079/bjn19790077>.
135. European Commission. Commission delegated regulation (EU) 2016/127. *Off J Eur Union*. 2016; 20
136. Pereira-da-Silva L, Pissarra S, Gomes A, Barroso R, Fernandes C, Virella D, et al. Guidelines for enteral nutrition in infants born preterm: 2023 update by the Portuguese Neonatal Society. Part I. Nutrient requirements and enteral feeding approach during the hospital stay. <http://dx.doi.org/10.24875/PJP.23000004>.
137. Harris JD QC, Manring MM, Siston RA, Flanigan DC. How to write a systematic review. *The American journal of sports medicine*. 2014; 42(11):2761-68. <https://doi.org/10.1177/0363546513497567>.

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5. Results and Discussion

Manuscript

A systematic review about long-chain polyunsaturated fatty acid supplementation in very preterm newborns: outcomes and safety

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[submitted to an international journal]

1. Introduction

Preterm birth, occurring before 37 weeks gestational age (GA), is categorized into late preterm (32 to 37 weeks), very preterm (28 to 32 weeks), and extremely preterm (less than 28 weeks) ⁽¹⁾. In 2020, approximately 15% of all preterm births were born before 32 weeks and the highest preterm birth rate was observed in southern Asia (13,2 %), emphasizing global disparities ⁽²⁾.

Whether occurring spontaneously or through medical interventions, preterm birth presents substantial challenges on a global scale ^(1, 3, 4). Prematurity is a leading cause of child mortality, under 5 years of age, and is also linked to short and long-term effects, such as physical (as inferior performance and delayed development), neurodevelopment (as cognition marked by lower IQ and learning disorder), and socioeconomic factors ^(1, 2, 5-7). The burden of preterm birth is particularly high in low- and middle-income countries, especially in Southeast Asia and sub-Saharan Africa, where more than half of the babies that are born within less than 32 weeks do not survive due to the lack of equipment, cost-effective care and basic care for infections and breathing difficulties ^(1, 2).

Preterm infants have specific nutritional needs, requiring personalized strategies based on their health condition and level of prematurity. The critical period for neurodevelopment is from 24 to 52- weeks post-conception and is marked by increased energy needs ^(8, 9). If an inadequate energy supply is provided, the body can mobilize fatty acids, including long-chain polyunsaturated fatty acids (LCPUFAs), to meet immediate energy needs, potentially compromising their role in fetal development ⁽¹⁰⁾.

During the third trimester of gestation and early postnatal life these LCPUFAs are rapidly and highly accumulated in neural tissues ⁽¹¹⁾. However, premature birth interrupts the critical period of lipid accumulation during pregnancy, necessitating the supplementation of fatty acids in the newborn's postnatal diet ^(11, 12).

Docosahexaenoic acid (DHA) and arachidonic acid (AA), the two primary LCPUFAs, are essential nutrients for premature infants, as they appear to play a pivotal role in the synthesis of the requisite quantities, for normal brain development ⁽¹³⁾. Numerous studies, especially biochemical ones, have shown that full-term infants receiving a full spectrum of LCPUFAs through breast milk have high concentrations of these fatty acids in their blood cells and higher levels of DHA in their brains compared to infants fed formulas without LCPUFAs ^(5, 7). Therefore, the current evidence indicates that including dietary LCPUFAs may be more beneficial for preterm infants.

Currently, there is insufficient evidence to support the use of LCPUFA in preterm infants, especially those who were born under 32 weeks GA or less. Although recent systematic reviews have studied the effects of LCPUFA in both term and preterm infants for specific outcomes, such as necrotizing enterocolitis and retinopathy of prematurity ^(14, 15), none have specifically studied very preterm infants supplemented with LCPUFA, born under 32 weeks of GA, and in terms of overall health outcomes. Therefore, the objective of this review was to evaluate the impact of LCPUFA supplementation on major primary outcomes, such as cognitive and neurodevelopmental, gastrointestinal, respiratory, visual, anthropometry and growth, and biomarkers and nutritional status, in very preterm infants born under 32 weeks of GA and LCPUFA supplemented.

This systematic review aimed to evaluate the association between enteral supplementation with high-dose LCPUFA during the neonatal period and the risk of health and developmental problems in very preterm infants born under 32 weeks of GA. In addition, this study aimed to determine whether there are doses higher than the established safety threshold that can be administered to preterm infants to improve health outcomes without causing harm.

2. Methods

2.1. Study design

This systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework ⁽¹⁶⁾, adhering to PRISMA's guidelines ⁽¹⁷⁾. Besides, it was pre-registered in advance on the International Prospective Register of Systematic Reviews (PROSPERO: CRD42023461646) ⁽¹⁸⁾.

2.2. Search strategy

The literature search used to respond to this systematic review objectives was performed from September 2023 to January 2024 within three databases, namely Medline (PubMed), Scopus, and Web of Science.

A search expression was created by the research team. The final search expression in Medline was the following one: ("premature new born" OR preterm* OR premature* OR "neonatal" OR "Low birth weight" OR "very low birth weight") AND ("long chain" OR "fatty acids" OR "PUFA" OR "lipids" OR "docosahexaenoic acid supplementation" OR "arachidonic acid supplementation" OR "omega-3" OR "omega-6") AND ("cognitive development" OR "visual development" OR "retinopathy" OR "pulmonary hypertension" OR "gut microbiota" OR gut* OR "immune system" OR "intestinal failure" OR "liver disease" OR "syndrome" OR "growth" OR "maturation" OR "development" OR "health" OR "safety dose" OR "tolerance" OR "tolerable Upper Intake Level" OR "Safe Upper Limit" OR "Maximum Tolerable Dose" OR "Acceptable Intake Level" OR "Tolerable Intake"). A similar expression was employed for the remaining two databases, with keywords discerned based on the collective knowledge of the research team.

After formulating the search string and identifying the database source, 20,746 articles were transferred to CADIMA, an open-access online tool developed for improving and presenting the evidence synthesis process and helping in effective reporting ⁽¹⁹⁾.

2.3. Eligibility criteria

Based on the question type PICOS (Population, Indicator, Comparator, Outcome, Study design), Table 1 outlined eligibility and exclusion criteria used to identify studies of interest ⁽²⁰⁾. Only articles written in English and Portuguese were included in this systematic review. Although the search did not impose specific study designs, reviews, abstracts-only, single-case studies, books, and conference papers were not included.

Table 1. Eligibility criteria of the studies included in this review according to the Population, Indicator, Comparator, Outcomes, and Study design (PICOS) format.

	Inclusion criteria	Exclusion criteria
P Population	Very premature infants, born with a gestational age of less than 32 weeks.	Premature infants born with a gestational age of 32 weeks or more. Infants born with health disabilities.
I Indicator *	(1) Modular supplementation of LCPUFA to increase energy density. (2) Enteral supplementation of LCPUFA.	Intravenous supplementation of LCPUFA.
C Comparator	Standard care.	
O Outcome	(1) Effects of supplementation with LCPUFA on the cognitive, gastrointestinal, respiratory, and visual development, and anthropometric growth and biomarkers status. (2) Higher doses than the established safety threshold of LCPUFA supplementation.	Adverse effects of excess and deficit LCPUFA supplementation.
S Study design	Original studies.	Reviews, abstracts (only), case studies of an individual, dissertations, theses, monographs, books, and conference articles.

* Indicators and outcomes are different for each objective (1 and 2 respectively)

2.4. Study selection and data extraction

After the data selection process, CADIMA and the reviewers identified and automatically eliminated 3,585 articles, considered as duplicates, as presented in Figure 1. Then the screening process involved two steps. Firstly, and using CADIMA, two independent reviewers applied the eligibility criteria and selected the studies of interest for inclusion in this systematic review, based on the title and abstract. In case of disagreement, a third research team member confirmed the process. Secondly, the main research of this systematic review extracted the data, by reading the full-text articles. The remaining team members participated in resolving any uncertainties encountered during the selection of identified studies. After, some of the references from the selected articles were reviewed to complement some relevant missing information, using the snowballing technique procedure ⁽²¹⁾.

During this process, a study was included if it addressed at least one of the two proposed objectives. Essentially, an article was considered for inclusion if it provided information about 1) the effect of preterm infants with a GA of less than 32 weeks supplemented with an LCPUFA formula or about the 2) higher doses than the established safety threshold of LCPUFA supplementation being considered without causing harm. Following this process, 223 studies were included in the final review (Figure 1). However, only articles that addressed the first objective were identified and included. The exclusion criteria for the studies are outlined in Table 1. The reviewers were not blinded to the journal titles or the names of the study authors. All necessary information to address the study objectives was already present in the published articles, obviating the need for additional contact with authors.

Table 2 was design to summarize the studies eligible for this systematic review and it has the following information: country, type of study, sample size, age at which the health outcome was evaluated, sex, gestational age, concentration, source and

duration of the LCPUFA supplementation, feeding type, existence (or not) of AA supplementation and parental nutrition (PN), description of the health outcomes associated with the long-chain polyunsaturated fatty acids supplementation in very preterm infants and the quality assessment score, providing an overview of the included studies accompanied by their detailed methodologies.

2.5. Quality assessment

To assess the quality of the studies included, we applied predefined tools. The methodological quality and potential biases of randomized control trials (RCTs) were evaluated using the Cochrane Collaboration revised tool designed for this purpose ⁽²²⁾. Additionally, the Newcastle-Ottawa Scale (NOS) was employed to appraise the quality of case-control studies, assigning an overall quality score ranging from 0 to 9 stars. Any discrepancies among reviewers were resolved through consensus ⁽²³⁾.

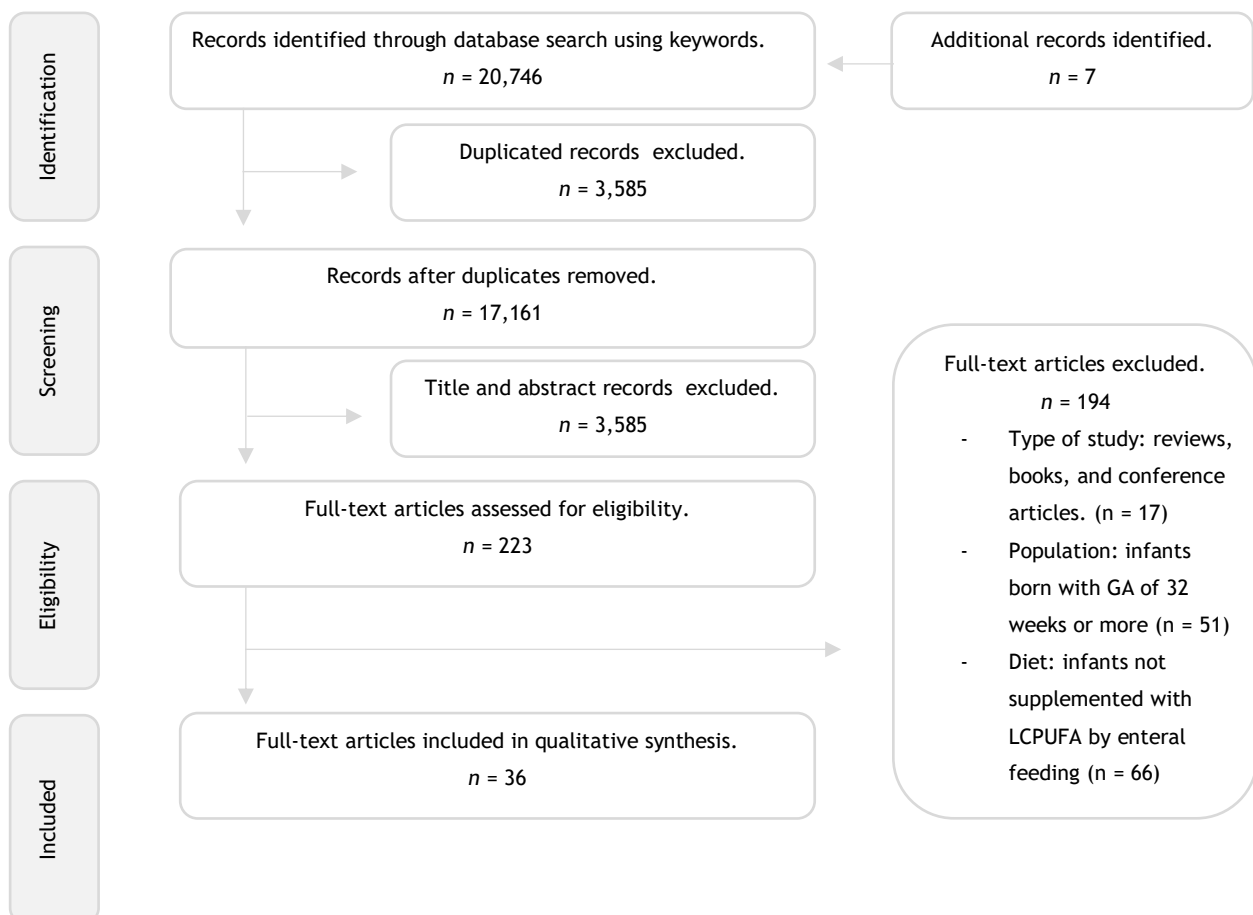


Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart.

3. Results

3.1. *Study selection and characteristics*

The selection procedure is summarized in Figure 1. Following a comprehensive screening process, 36 full-text articles were analysed and detailed in Table 2, comprising 26 RCTs and 10 case-control studies. As a whole, the studies involved 5,753 subjects, ranging from 31 weeks corrected age (CA) ^(24, 25) to 8 years old ⁽²⁶⁻²⁸⁾. Twelve articles were published between 1992 and 2010, and 24 articles were published between 2010 and 2023. Geographically, the distribution of studies was as follows: 17 studies were conducted in Europe, 14 in America, three in Oceania ⁽²⁹⁻³¹⁾, one in Asia ⁽³²⁾, and another in Africa ⁽³³⁾. It is noteworthy that a considerable number of studies were conducted in Nordic countries. Of these, Sweden contributed with four articles ⁽³⁴⁻³⁷⁾, and Norway with six articles ^(26, 27, 38-42). Additionally, 10 studies were conducted in the United States of America (USA) ^(28, 43-51).

Regarding the quality assessment of the studies included, the mean (standard deviation, SD) quality of the 10 case-control studies included was 5.2 (3.1) points, ranging from a minimum of four ^(13, 38, 48, 52, 53) to a maximum of nine ⁽⁴²⁾ points. Among the RCTs, 22 studies presented a low risk of bias, and four articles ^(30, 31, 40, 49) had some concern regarding bias.

3.2. *Gestational age and Long-chain polyunsaturated fatty acids description*

The mean (SD) of the gestational age of the participants included in this review was 28.3 (8.3) weeks, with a minimum of 20 weeks ⁽³⁷⁾, and a maximum of 32 weeks ⁽⁵⁴⁾. The duration of LCPUFA supplementation varies considerably, from 10 days ⁽⁵⁴⁾ to 14 months ⁽⁵⁵⁾, typically initiated shortly after birth.

As represented in Figure 2, the newborns were fed in different ways, namely with formula (n = 7 articles), human milk (HM) or donor human milk (DHM) (n = 13), human milk and formula (n = 8), and human milk or formula (n = 8).

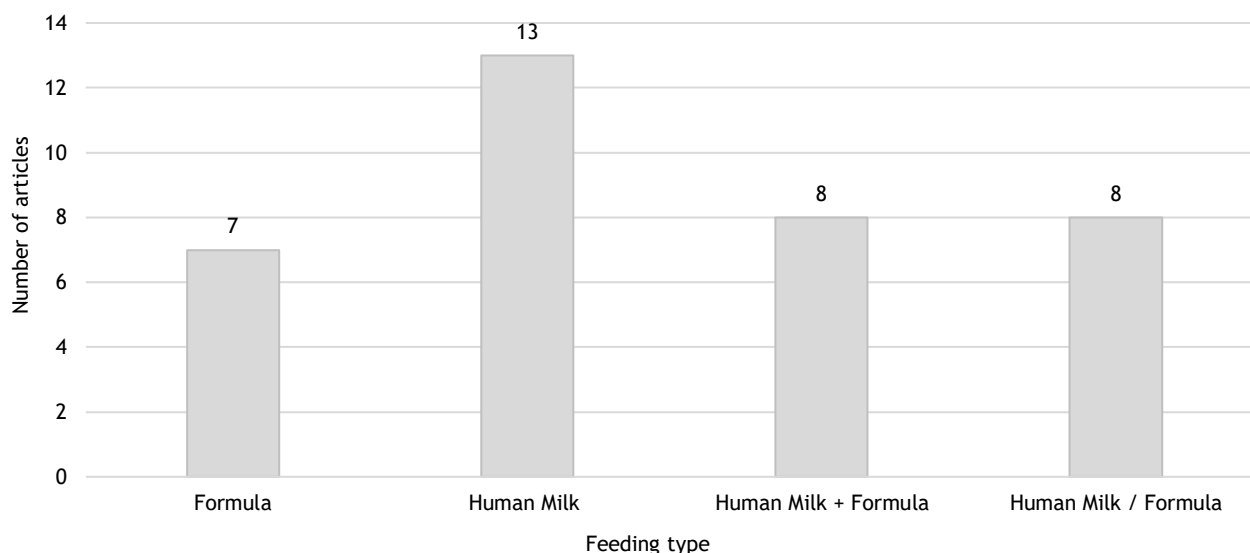


Figure 2. Preterm infant's feeding type identified in the literature.

As this study focuses on LCPUFA supplementation, it is important to highlight that, DHA is supplemented alone in 14 articles, and complemented with AA supplementation in 22 articles. The sources of supplementation included soy oil emulsions, used as placebo in six studies (27, 29-31, 38, 42) and as an intervention in another six studies (26, 27, 38, 42, 48, 51), fish oil in five studies (43, 47, 49, 50, 54), egg lipid extracts in four studies (40, 47, 49, 56), fungi and microalgae oils in four studies (34-36, 57), evening primrose oil in two studies (40, 56), human milk fortifiers in two studies (32, 58), and algal oil in two studies (24, 25).

Parental nutrition was also a part of the first day's intervention in 16 studies (44,4%), while 20 studies (55,5%) did not include it.

3.3. Impact of feeding type on health outcomes

A variety of health outcomes were assessed across the studies, as shown in Figure 3. Cognitive and neurodevelopmental outcomes were reported in ten studies, visual outcomes in eight studies, respiratory outcomes in four studies, gastrointestinal outcomes in two studies, growth and anthropometry in eight studies, and biomarkers and nutritional status in nine studies.

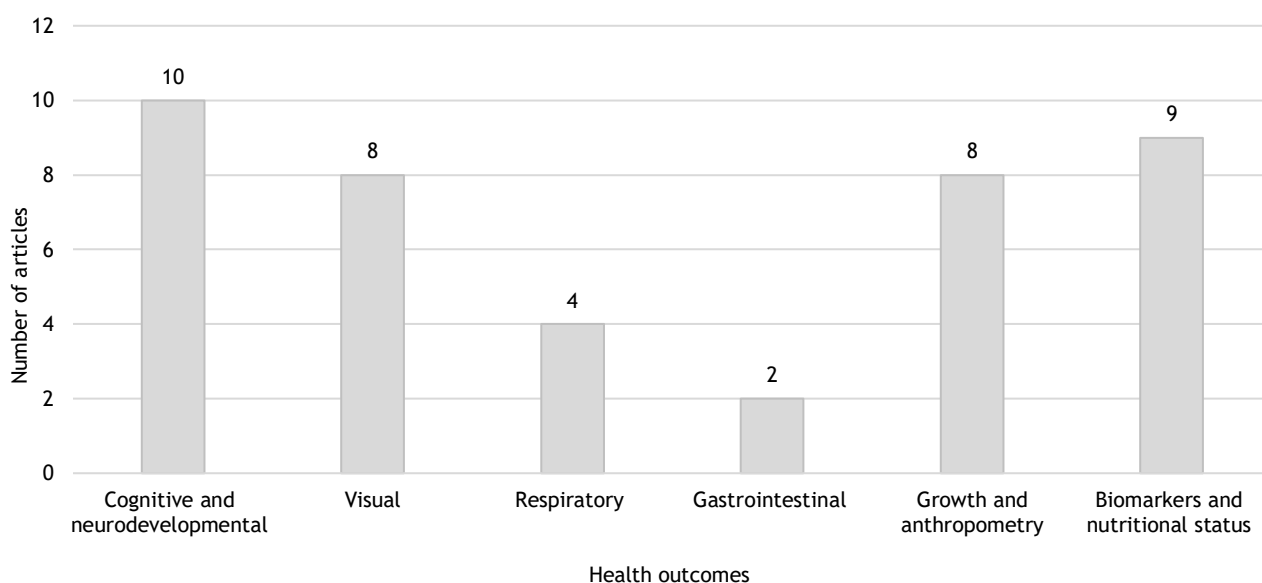


Figure 3. Preterm infant's health outcomes identified in the literature.

3.3.1. Cognitive and neurodevelopmental outcomes

In general, infants who received higher levels of DHA exhibited superior neurodevelopmental outcomes in eight of the ten studies that investigated this association. The primary outcomes included were behaviour acuity in six studies ^(26, 30-32, 43, 49), full scale IQ in three studies ^(27, 30, 31), visual evoked potential in two studies ^(37, 49), and attention tests in two studies ^(32, 42). Problem-solving skills, and cerebral white matter injury were only studied in one study each ^(26, 38).

The results of three studies indicated that infants who were fed HM or formula and who exhibited higher levels of DHA demonstrated superior behavioural acuity outcomes. These outcomes included higher behaviour rating scale scores at 18 months

CA, reduced sensory sensitivity behaviours at 4 months CA, and higher behavioural acuity scores at 3 months CA ^(32, 43, 49). Boone et al.⁽⁴³⁾ also reported positive outcomes related to higher levels of DHA, including higher neurological thresholds and lower distractibility and hyperactivity at 38 months CA. Nevertheless, in three studies, infants who were fed with HM at the ages of 5, 7, and 9 years old did not exhibit significant behavioural disorders, regardless of whether they were DHA-supplemented or not ^(26, 30, 31).

Regarding full-scale IQ level (FSIQ), DHA concentration was a significant predictor of higher values of this outcome at 8 years old, suggesting potential long-term benefits of DHA:AA supplementation on cognitive development in one article with including HM-fed infants ⁽²⁷⁾. Also, Gould et al.⁽³⁰⁾ observed that similar-fed infants with higher levels of DHA had slightly higher FSIQ scores at 5 years old. However, Collins et al.⁽³¹⁾ found out that 7-years-old infants with higher levels of DHA did not differ in FSIQ.

Higher levels of DHA and AA were identified as significant predictors of superior flash visual evoked potential (VEP) responses, indicating enhanced cognitive and visual development at six months in infants fed with formula and supplemented with DHA:AA (based on egg derived triglyceride or fungal oil + fish oil) ⁽⁴⁹⁾. However, van Wezel-Meijler et al.⁽³⁷⁾ did not identify any significant differences between supplemented and non-supplemented infants regarding VEP responses.

Two studies have demonstrated a correlation between higher levels of DHA, and enhanced performance on attention test outcomes, including enhanced orientation and motor quality ^(32, 42). However, Westerber et al.⁽⁴²⁾ observed no significant differences in Mental Development Index and Ages and Stages Questionnaire scores between infants.

In a study by Henriksen et al.⁽³⁸⁾, it was found that infants fed with HM and supplemented with DHA and AA exhibited improved problem-solving skills at six months

CA. In contrast ⁽²⁶⁾ did not observe significant cerebral white matter effects in 8 years old infants fed with HM and supplemented with DHA:AA.

3.3.2. Visual Outcomes

In general, infants who received higher levels of DHA exhibited superior visual outcomes in seven out of the eight studies that investigated this association. The primary visual outcomes examined were visual acuity in four studies ^(36, 37, 45, 49) and retinopathy of prematurity (ROP) in three studies ^(25, 34, 35). Only one studied examined retinal development ⁽⁵¹⁾. Although VEP are also considered as a visual outcome, they are described in the context of cognitive and neurodevelopmental outcomes ^(37, 49).

In terms of visual acuity, higher levels of DHA were associated with more favourable outcomes in infants fed with HM or formula and DHA:AA supplemented ^(36, 49). However, van Wezel-Meijler et al. ⁽³⁷⁾ found that, in infants fed with formula, visual acuity exhibited a slightly, but not statistically significant, in infants supplemented with DHA:AA, compared to unsupplemented infants. The findings of S. E. Carlson et al. ⁽⁴⁵⁾ study at four months CA were consistent with those of the previous studies.

Infants fed with HM or DHM and supplemented with DHA:AA exhibited a significantly lower incidence of severe retinopathy of prematurity (ROP) compared to those who were not supplemented, with a 50% reduction in the incidence of this outcome ^(25, 34, 35). Bernabe-García et al. ⁽²⁵⁾ also found that DHA was associated with a reduced risk of eye complications such as blindness in a study where infants were fed with HM or formula. In the study by Hellström et al. ⁽³⁴⁾ higher levels of AA were not found to be associated with ROP, while lower levels of eicosadienoic acid remained associated with severe ROP.

Regarding retinal development, lower levels of DHA were associated with higher rod thresholds at 36 weeks CA, particularly in infants fed with formula. However, these

differences were not observed at 57 weeks, when comparing with infants fed with HM and supplemented formula ⁽⁵¹⁾.

3.3.3. Respiratory Outcomes

The findings of this systematic review indicate that infants who were fed with HM generally exhibited superior respiratory outcomes compared to those who were fed with formula, particularly when AA were included.

Regarding bronchopulmonary dysplasia (BPD), infants fed with HM and supplemented with DHA:AA, were found to have no significant incidence of this outcome, comparing to those unsupplemented ^(34, 41). Otherwise, infants fed with formula and supplemented with DHA:AA appear to have a lower, although not statistically significant, incidence of BPD ^(29, 45). Wendel et al. ⁽⁴¹⁾ conducted a study investigating the effects of HM or DHM fed infants supplemented with DHA:AA. Their findings indicated that these infants exhibited better respiratory outcomes, with fewer days of respiratory support and lower oxygen requirements compared to non-supplemented infants. It is noteworthy that at 40 weeks GA, this author found no significant differences in the incidence of BPD between infants who were supplemented with DHA and AA and those who were not. In the study by Collins et al. ⁽²⁹⁾, no significant differences were observed between groups of infants fed with HM or formula. However, the severity of BPD was found to be lower in the DHA-supplemented group.

3.3.4. Gastrointestinal outcomes

The two studies of gastrointestinal outcomes reviewed in this article, suggest that infants fed with human milk or formula supplemented with DHA:AA, had better outcomes. Regarding necrotizing enterocolitis (NEC), infants supplemented with DHA:AA and DHA alone had a lower incidence of NEC over a period of 14 days in both studies ^(24, 33). Additionally, higher concentrations of DHA:AA in infants were associated

with lower levels of interleukin-1b (IL-1b), indicating a potential anti-inflammatory effect.

3.3.5. Growth and Anthropometry Outcomes

Overall, infants who were fed with HM, exhibited similar growth outcomes between those who were supplemented with DHA and those who were not. The primary growth and anthropometric outcomes included in this review were anthropometric data in eight studies ^(27, 39, 40, 44, 45, 47, 50, 53) and postnatal growth in one study ⁽³⁹⁾.

In four studies, anthropometric data demonstrated that at 8 years, 3 months, 92 weeks, and 20 months respectively, there were no observed differences in physical parameters such as weight, length, head and mid-arm circumference, and weight-for-age or length-for-age z-scores between those who were supplemented with DHA:AA and those who were not ^(27, 40, 42, 53). However, only one observed superior linear growth, from birth to term-equivalent age, in infants with higher levels of DHA ⁽⁴⁰⁾. Conversely, another study observed that infants fed with HM or formula and supplemented with DHA and AA demonstrated an increase in lean body mass and a decrease in fat mass at 12 months of age, compared to unsupplemented infants ⁽⁴⁷⁾. Regarding sex differences, only one study indicated that higher levels of DHA resulted in lower gains in weight, length, and head circumference, as well as fat-free mass, in males. However, no significant differences were observed in females ⁽⁵⁰⁾. Finally, Carlson et al. ^(44, 45) observed that, infants fed with formula and with higher DHA levels exhibited negative anthropometric data in their first-year growth, as evidenced by lower Z-scores for weight, length, and head circumference.

Higher levels of DHA had improved nutritional status and growth outcomes, reducing the risk of postnatal growth. These results were observed in the study by Moltu et al. ⁽³⁹⁾, in which infants received HM or DHM fortified with DHA:AA.

3.3.6. Biomarkers and Nutritional Status

The effects of human milk and formula feeding on various biomarkers and nutritional status in preterm infants are distinct. The primary visual outcomes examined were plasma composition in four studies ^(46, 48, 52, 54) and red blood cell (RBC) in three studies ^(28, 52, 58). Only one study each examined mineral balance, vitamin E status, and plasma oxidative status ^(56, 57, 59).

Infants fed with HM and formula and DHA supplemented formula showed a plasma composition with higher DHA concentrations at 8-, 36- and 57-weeks CA, and 6 months, in three studies ^(46, 48, 52). Bernhard et al. ⁽⁵⁴⁾ demonstrated that preterm formula supplemented with DHA and choline, increased plasma choline concentrations to near-term infants' levels, thereby promoting DHA homeostasis in these preterm infants.

Higher levels of DHA were associated with an increase in red blood cell (RBC) phospholipid DHA content, without a reduction in AA levels, in infants fed with HM or supplemented DHA formula, compared to infants fed with an unsupplemented formula ^(52, 58). Robinson et al. ⁽²⁸⁾ and Lapillone et al. ⁽⁵²⁾ also observed that infants fed with formula with a lower (DHA 20 mg/kg/d + AA 40 mg/kg/d) or no DHA:AA concentration did not change RBC phospholipids DHA levels and AA levels remain stable.

In a study by F. E. Martinez ⁽⁵⁶⁾ about mineral balance, it was found that higher DHA:AA levels were associated with increased urinary phosphorus excretion. However, this correlation was not statistically significant when other minerals, such as calcium, phosphorus, magnesium, zinc, or copper, were considered.

Regarding vitamin E status, higher DHA:AA levels in formula-fed infants were found to be associated with higher levels of alpha-tocopherol (vitamin E) and gamma-tocopherol, indicating an improvement in antioxidant activity and protection against oxidative stress-related diseases ⁽⁵⁹⁾.

Ramiro-Cortijo et al. ⁽⁵⁷⁾ observed that incorporating DHA:AA into HM altered the plasma fatty acid profile and enhanced antioxidant activity, potentially improving plasma oxidative status and health benefits.

3.4. Safety in adherence to ESPGHAN Guidelines

Four of the 36 articles included reported values above the ESPGHAN guidelines. Among these, positive outcomes were observed in gastrointestinal outcomes ^(24, 33), visual outcomes ⁽²⁵⁾, and biomarkers and nutritional status outcomes ^(28, 46). Furthermore, no adverse effects were reported, or significant differences were found between infants supplemented with LCPUFA in any of the studies.

4. Discussion

This review has demonstrated the impact of LCPUFA supplementation and feeding type on the health outcomes of preterm infants, addressing two primary objectives. Firstly, it explores how neurodevelopmental, cognitive, gastrointestinal, respiratory, visual acuity, growth patterns, and nutritional status outcomes are influenced by feeding preterm infants with HM or formula supplemented with DHA, with or without additional AA supplementation. To address the second objective - determining if there are higher doses than the established safety threshold of LCPUFA supplementation without causing harm - the studies reviewed did not provide clear answers. This lack of clarity is due to variability in study designs, differences in LCPUFA formulations, and the range of doses examined. Additionally, heterogeneity in health outcomes and assessment ages further complicated findings. This gap underscores the need for more standardized and comprehensive studies to fully understand LCPUFA supplementation's impact.

4.1. Feeding type and health outcomes

Feeding type significantly impacts infant health, with HM or DHM demonstrating substantially better outcomes, particularly with LCPUFA supplementation like DHA and AA. HM is the gold standard due to its immunological, gastrointestinal, and neurodevelopmental benefits, effectively promoting cognitive and neurodevelopmental outcomes in preterm infants, even without additional supplementation, and protecting against conditions like late-onset sepsis, ROP, and NEC ⁽⁶⁰⁻⁶³⁾. However, some studies reviewed did not consistently observe these benefits without DHA or AA supplementation ⁽⁶²⁾.

4.2. Parenteral nutrition (PN) and health outcomes

According to the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) guidelines, it is recommended that all infants receive PN until the third day of life, but it may be initiated as early as the first day of life until they can tolerate enteral feeding ⁽⁶⁴⁾. In this review, infants who receive early PN feeding and LCPUFA supplementation seem to exhibit improved better outcomes, as respiratory, anthropometric and growth, when compared to those who did not receive PN feeding. Studies have shown that early aggressive PN positively influences health outcomes, leading to better tolerance of enteral nutrition, higher total caloric intake, and a reduction in the number of days spent in the neonatal intensive care unit ⁽⁶⁵⁾.

4.3. Duration of LCPUFA supplementation

In this review, the duration of LCPUFA supplementation varied from 10 days to 14 months. While current evidence does not strongly support the impact of supplementation duration on reducing complications, it may still be an influencing factor. Our results did not show a consistent pattern. In the Bernhard et al. study, 10 days of DHA supplementation increased plasma choline and betaine levels, suggesting short-term benefits but no significant long-term impact ⁽⁵⁴⁾. Conversely, Tanaka et al.

study, with 12 months of supplementation, showed improvements in cognitive and neurodevelopmental outcomes, such as sustained attention and emotional regulation, suggesting longer durations may be necessary for significant benefits ⁽³²⁾. Therefore, while the duration of LCPUFA supplementation is crucial, more research is needed to determine the optimal length for the best health outcomes in preterm infants.

4.4. LCPUFA supplementation and health outcomes

This systematic review found that higher DHA levels improve early developmental behavioural acuity and correlate with better FSIQ and VEP scores, suggesting potential cognitive benefits ^(27, 30, 31, 37, 49). However, discrepancies between studies highlight the need for further research, considering factors like infant age, feeding type, and study quality. Additionally, DHA and AA supplementation showed superior cognitive performance, though there is no consensus on the optimal dose or long-term effects ⁽⁶⁶⁾. Another review found no significant differences in visual acuity, neurodevelopment, or physical growth with LCPUFA supplementation in formula-fed infants ⁽⁶⁷⁾.

In this systematic review, NEC was the primary gastrointestinal outcome studied, and its incidence was notably lower in preterm infants supplemented with DHA alone or with AA, indicating a potential protective and anti-inflammatory effect ^(24, 33). This finding aligns with ⁽¹⁴⁾, who reported that DHA with AA significantly reduces NEC risk, though DHA alone might increase the risk, warranting further investigation.

Regarding BPD, no significant difference in BPD incidence was observed in infants fed HM, with or without DHA supplementation in this review ^(29, 34, 41, 45). However, a non-significant reduction in BPD incidence was noted in formula-fed infants with higher DHA levels. While DHA did not conclusively reduce BPD incidence, it showed potential benefits in improving respiratory support and lowering oxygen needs. These findings contrast with previous reviews suggesting no protective effect of DHA against BPD, especially in infants with a gestational age of less than 32 weeks ^(68, 69). Given that only

four studies addressed this outcome, more research is needed for definitive conclusions.

This review indicates that DHA supplementation positively impacts visual outcomes in preterm infants, with higher DHA levels consistently associated with improved ROP outcomes and visual acuity, particularly in those fed HM or DHA formula (25, 34-36, 51). Studies highlight DHA's protective role against severe eye complications such as blindness, and while lower DHA levels were linked to impaired retinal development, this effect diminished by 57 weeks GA, emphasizing the importance of early DHA intervention (25, 34). Additionally, Diggikar et al. (15) found that LCPUFA supplementation did not reduce overall ROP incidence but did reduce severe ROP, aligning with our review's findings. Therefore, DHA supplementation plays a crucial role in enhancing visual outcomes and reducing severe ROP in preterm infants, though further research is needed to confirm these benefits and establish definitive conclusions.

The studies reviewed provide a nuanced understanding of DHA supplementation's impact on growth and anthropometric outcomes in preterm infants. Infants fed with HM showed comparable growth in weight, length, head circumference, and z-scores for weight-for-age and length-for-age, regardless of DHA supplementation, suggesting that HM alone supports adequate growth (27, 40, 42, 53). However, infants fed formula with higher DHA levels exhibited poorer growth outcomes, including lower z-scores for weight, length, and head circumference, potentially due to marine oils slowing weight gain during rapid growth and the absence of AA supplementation (44, 45). The absence of AA supplementation in these formula-fed infants may be significant, as one study found a positive correlation between AA and growth in preterm infants fed standard formula (70). Additionally, gender-based differences were observed, with higher DHA levels linked to reduced growth in male infants but not females, though this

finding is based on a single study ⁽⁵⁰⁾. A complementary review also found that maternal DHA supplementation during lactation positively affected female infants' weight and growth velocity, while negatively impacting males ⁽⁷¹⁾. These findings highlight the need for further research on sex-specific responses to DHA supplementation and the importance of balanced nutrient formulations in infant growth.

This systematic review reveals that DHA supplementation in preterm infants significantly improves biomarkers and nutritional status by increasing DHA levels in plasma and red blood cells and stabilizing AA levels ^(46, 48, 52, 54, 58). Bernhard et al. ⁽⁵⁴⁾ found that DHA and choline supplementation achieved plasma choline levels comparable to term infants, supporting DHA homeostasis. F. E. Martinez et al. ⁽⁵⁶⁾ noted an increase in urine phosphorus loss with elevated DHA levels, though this was not statistically significant, indicating a need for further research on mineral balance. Additionally, higher DHA levels improved vitamin E status, enhancing antioxidant defences against oxidative stress ⁽⁵⁹⁾. Ramiro-Cortijo et al. ⁽⁵⁷⁾ demonstrated that DHA supplementation altered the plasma fatty acid profile and boosted antioxidant defences, highlighting the overall benefits of DHA supplementation.

4.5. *Safety considerations*

Regarding safety considerations, none of the reviewed studies reported adverse effects from exceeding ESPGHAN guidelines for DHA supplementation, indicating a generally favourable safety profile. The lack of reported adverse effects despite higher doses underscores the robustness of the safety protocols in these interventions. Specifically, higher daily intakes did not lead to adverse reactions but rather demonstrated beneficial effects on gastrointestinal, visual, and nutritional outcomes. These findings suggest that the studied supplementation strategies are safe, even at levels exceeding ESPGHAN recommendations, though further research is needed to

explore the long-term effects and broader clinical applicability of these safety protocols.

4.6. *Final remarks*

This systematic review is the first to comprehensively examine health outcomes in preterm infants born before 32 weeks of gestation who received LCPUFA supplementation. However, several limitations should be noted. Firstly, 10 of the 36 studies were cross-sectional, which hinders the ability to establish causal relationships and weakens the associations between LCPUFA supplementation and health outcomes. Additionally, the limited overlap in outcomes among the studies and the overall quality variability suggest that more prospective cohort studies and intervention trials are needed. Secondly, the fragile nature of preterm infants and extrinsic factors, such as the age at which exclusive human milk or formula feeding is recommended, could impact outcomes. Despite these limitations, this review underscores the importance of adequate nutrition for preterm infants, highlighting the crucial role of LCPUFA supplementation and human milk feeding, especially for very preterm infants. It identifies a key area for medical advancement to address both short- and long-term issues related to preterm birth. By clarifying the nutritional needs of preterm infants, this review advances healthcare practices aimed at improving health outcomes and quality of life for these vulnerable populations.

5. Conclusion for Practice

This review suggests that DHA and AA supplementation can improve health outcomes for preterm infants born before 32 weeks of gestation, enhancing cognitive, neurodevelopmental, gastrointestinal, respiratory, visual, and growth outcomes, as well as biomarkers and nutritional status. Human milk remains the preferred feeding method, and current evidence supports the benefits of including LCPUFAs in clinical

practice. However, further research is needed to determine the optimal supplementation strategies and explore the long-term effects of early LCPUFA intervention.

Table 2. Characteristics of the studies included concentration and source formula and associated health outcomes in very preterm infants.

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Collins et al. ⁽²⁸⁾ 2017, Australia	RCT	n = 1273 36 weeks 53,4 % males	26,7 weeks	36 weeks (starting 3 days after birth)	(1) Placebo Neonatal care with soy oil emulsion (2) Neonatal care with DHA emulsion (60 mg/kg/day of DHA)	No	HM or formula	No	Respiratory outcomes Bronchopulmonary dysplasia (BPD)	In group 2, the incidence of BPD was not significantly reduced compared to group 1. Mild BPD severity was significantly lower in group 2 compared to group 1.	Low risk ¹
Collins et al. ⁽³⁰⁾ 2015, Australia	RCT	n = 604 7 years 53,6 % males	30,0 weeks	36 weeks (starting 3 days after birth)	(1) Placebo Neonatal care with soy oil emulsion (2) Neonatal care with DHA emulsion (60 mg/kg/day of DHA)	No	HM or formula	No	Cognitive and neurodevelopmental outcomes Full scale IQ (FSIQ), Behaviour acuity	The FSIQ did not differ significantly between groups 1 and 2. At 7 years old, parental reports showed no significant differences between groups in executive functioning, ADHD symptoms, emotional and behavioural problems, general health status and quality of life.	Some concerns ¹
Gould et al. ⁽²⁹⁾ 2022, Australia	RCT	n = 656 4,9 years 52,7 % males	26,8 weeks	36 weeks (starting 3 days after birth)	(1) Placebo Neonatal care with soy oil emulsion (2) Neonatal care with DHA emulsion (60 mg/kg/day of DHA)	No	HM or formula	No	Cognitive and neurodevelopmental outcomes Full scale IQ (FSIQ), Behaviour acuity	Group 2 had slightly higher FSIQ scores at 5 years old, suggesting better cognitive function compared to group 1. The incidence of clinical disorders (e.g., autism spectrum disorder, ADHD, other behavioural or neurologic disorders) was below 7% and similar between groups.	Some concerns ¹
Martinez et al. ⁽⁵⁵⁾ 2002, Brazil	Case-control study	n = 44 34 weeks 50 % males	31 weeks	30 days	(1) Placebo Preterm formula (2) Preterm formula with LCPUFA (egg lipid extracts + evening primrose oil)	Yes	Formula	Yes	Biomarkers and Nutritional Status Mineral balance	Group 2 exhibited higher urine phosphorus loss compared to group 1. There were no significant disparities in mineral balance (Ca, P, Mg, Zn, or Cu) between the two groups.	4 ²

(continued)

Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Vanderhoof et al. ⁽⁵²⁾ 2000, Columbia	Case-control study	n = 288 92 weeks 53,1% males	30,6 weeks	92 weeks	(1) Human milk (2) Placebo Preterm formula (Premie SMA) (3) Supplemented formula with LCPUFA (Premie SMA). Until 40 weeks, 24 cal/oz of formula and until 48 weeks 20 cal/oz. At 92 weeks, all groups received 20 cal/oz of term formula (no LCPUFA).	Yes	HM and formula	No	Growth and anthropometry outcomes Anthropometric data	At 40 weeks of follow-up, group 2 had higher weight, length, head and midarm circumference, compared to groups 1, 3. However, at 92 weeks there were no differences in these physical parameters. At 40 weeks CA, group 3 had significantly greater concentrations of all 10 phospholipid fatty acids variables, compared to other groups. At 92 weeks CA, group 1 had greater DHA levels than group 3.	4 ²
El Fadl et al. ⁽³²⁾ 2021, Egypt	RCT	n = 60 37 weeks 35,0 % males	31 weeks	14 days or discharge	(1) Placebo Neonatal care (2) Neonatal care with DHA (100 mg/kg/day of DHA)	No	HM or formula	No	Gastrointestinal outcomes Necrotizing enterocolitis (NEC)	Group 2 had lower incidence of clinically diagnoses of NEC and staging on day 10 compared to group 1. Group 2 showed a decrease in IL-1b levels, indicating a potential anti-inflammatory effect, compared to group 1. Neonatal Intensive Care Unit length of stay was significantly shorter in group 2. No significant difference between the groups regarding changes in haemoglobin.	Low risk ¹

(continued)

Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Lapillonne et al. ⁽⁵¹⁾ 2000, France	Case-control study	n = 33 6 months	29,4 weeks	6 months	(1) Human milk (2) Placebo Preterm formula (3) Preterm formula with DHA [high-DHA and low-EPA; ratio 4.8:1] Starting at 4 months, all infants were fed with term formula.	No	HM and formula	No	Biomarkers and Nutritional Status Red blood cell (RBC), Plasma composition	Group 2 experienced a continuous decrease in RBC phospholipid DHA content, while AA content remained stable. Groups 1 and 3 had significantly higher DHA content, along with increased eicosapentaenoic acid (EPA) content and a decrease in AA content.	4 ²
Billeaud et al. ⁽⁵⁷⁾ 2018, France	RCT	n = 153 32 weeks 47,7 % males	28,8 weeks	Until 21 days or discharge (starting 1 day after birth)	(1) Placebo Human milk with 5g/100ml of Fat free HM fortifier [cHMF] (2) Human Milk with 4g/100ml of Fat HM fortifier [nHMF]	Yes	HM	No	Biomarkers and Nutritional Status Red blood cell (RBC)	Group 2 enhanced the assimilation of ALA and DHA fatty acids into plasma into plasma triacylglycerol and red blood cell phosphatidylethanolamine, respectively, without reducing arachidonic acid levels. Supplementation in group 2 led to an increased incorporation of <i>n</i> -9 long-chain monounsaturated fatty acids in red blood cells.	Low risk ¹
Kaempf-Rotzoll et al. ⁽⁵⁸⁾ 2003, Germany	RCT	n = 31 36 weeks 45,2 % males	30 weeks	42 days (starting 4 to 12 hours after birth)	(1) Human milk with a FM 85/Nestlé® supplement (2) Placebo Preterm formula based on palm seed and coconut oil (3) Preterm formula with LCPUFA based on egg lipids and evening primrose oil	Yes	HM and formula	Yes	Biomarkers and Nutritional Status Vitamin E	Supplementation in group 3 resulted in enhanced weight gain compared to the other groups. Group 3 exhibited higher levels of alpha-tocopherol (vitamin E) and gamma-tocopherol, leading to improved antioxidant status and protection against oxidative stress-related diseases.	Some concerns ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Bernhard et al. ⁽⁵³⁾ 2020, Germany	RCT	n = 24 33 weeks	32 weeks	10 days	(1) Placebo Preterm formula (2) Preterm formula with choline (30mg/kg/day) (3) Preterm formula with DHA emulsion fish oil-based (60 mg/kg/day) (4) Preterm formula with DHA (60 mg/kg/day) and choline (30 mg/kg/day)	No	Formula	No	Biomarkers and Nutritional Status Plasma composition	Supplementation in group 2 increased plasma choline and betaine levels to near-fetal concentrations. Choline supplementation (30 mg/kg/d), as in group 4, increases plasma choline to near fetal concentrations, dilutes the D9-choline tracer via increased precursor concentrations and improves DHA homeostasis in preterm infants.	Low risk ¹
Tanaka et al. ⁽³¹⁾ 2013, Japan	RCT	n = 24 18 months 45,8 % males	29,1 weeks	12 months	(1) Placebo Neonatal care (2) Neonatal care with Sphingomyelin-milk (SM) fortifier	No	HM or formula	No	Cognitive and neurodevelopmental outcomes Behaviour acuity, attention test	Percentage of sphingomyelin was higher in group 2 at 4, 6 and 8 weeks of age. Group 2 was significantly better on the sustained-attention test. Group 2 demonstrated significantly higher orientation and emotional scores on the Behaviour Rating Scale (BRS) as well as higher motor quality at 6, 12, 18 months.	Low risk ¹
Bernabe-García et al. ⁽²⁴⁾ 2019, Mexico	RCT	n = 110 31 weeks 48,2 % males	30 weeks	14 days	(1) Placebo Neonatal care with high oleic sunflower oil (2) Neonatal care with DHA rich algal oil (75 mg/kg/day)	No	HM or Formula	Yes	Visual outcomes Retinopathy of Prematurity (ROP)	Group 2 showed a lower risk of apnoea and developing stage 3 ROP, associated with a reduced risk of serious eye complications, such as blindness. There was no difference between groups 1, 2 in the incidence or risk of intraventricular haemorrhage or gastric bleeding.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Bernabe-Garcia et al. ⁽²³⁾ , 2021, Mexico	RCT	n = 214 31 weeks 49,5 % males	30 weeks	14 days	(1) Placebo Neonatal care with high oleic sunflower oil (2) Neonatal care with DHA rich algal oil (75 mg/kg/day)	Yes	HM or Formula	Yes	Gastrointestinal outcomes Necrotizing enterocolitis (NEC)	The incidence of confirmed NEC was significantly lower in group 2 compared to group 1. Being in the group 1 was the strongest predictor for developing NEC. No differences in adverse events (like mortality rates) between groups.	Low risk ¹
Moltu et al. ⁽³⁸⁾ , 2014, Norway	RCT	n = 44 36 weeks 63,6 % males	28,3 weeks	4 weeks (starting 1 day after birth)	(1) Placebo Human milk (2) Human milk with DHA (60 mg/kg/day) and AA (60 mg/kg/day)	Yes	HM or DHM	Yes	Growth and anthropometry outcomes Postnatal growth, Anthropometric data	Postnatal growth restriction reduced in group 2 infants, compared to group 1. Group 2 led to improved nutritional status and growth outcomes, reducing the risk of postnatal restriction.	Low risk ¹
Westerberg et al. ⁽⁴¹⁾ 2011, Norway	Case-control study	n = 92 20 months 52,2% males	28,7 weeks	9 weeks (starting 1 week after birth)	(1) Placebo Human milk fortified with 0.5 ml of soy oil and medium-chain triglyceride per 100 ml (2) Human milk fortified with 0.5 ml of soy oil per 100 ml (32 mg of DHA and 31 mg of AA)	Yes	HM or DHM	No	Cognitive and neurodevelopmental outcomes Attention test	Group 2 had higher plasma levels of DHA and AA and improved attention levels during free-play sessions compared to group 1. Moderate correlation between plasma DHA levels and cognitive development. No significant differences in Mental Development Index and Ages and Stages Questionnaire scores between groups.	9 ²
Almaas et al. ⁽²⁵⁾ 2016, Norway	RCT	n = 98 8,6 years 51 % males	26 weeks	9 weeks or discharge (starting 2 days after birth)	(1) Placebo Human milk fortified with 0.5 ml of placebo oil per 100 ml (2) Human milk fortified with 0.5 ml of soy oil per 100 ml (32 mg of DHA and 31 mg of AA)	Yes	HM or DHM	No	Cognitive and neurodevelopmental outcomes Behaviour acuity, Cerebral white matter injury	No significant behavioural or cerebral white matter effects were detected at 8 years of age.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration of supplementation (initial age)	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Henriksen et al. ⁽³⁷⁾ 2008, Norway	Case-control study	n = 129 6 months 54,6 % males	28,4 weeks	9 weeks (starting 2 days after birth)	(1) Placebo Human milk fortified with 0.5ml of soy oil and medium-chain triglyceride per 100 ml (2) Human milk fortified with 0.5 ml of soy oil per 100 ml (32 mg of DHA and 31 mg of AA)	Yes	HM or DHM	Yes	Cognitive and neurodevelopmental outcomes Problem-solving skills	Group 2 resulted in increased DHA and decreased AA levels in plasma. While there were no significant differences observed in adverse events or growth parameter, improved problem-solving skills, and altered brain responses to visual stimuli were observed in group 2 at 6 months follow-up.	4 ²
Henriksen et al. ⁽²⁶⁾ 2016, Norway	RCT	n = 98 8.6 years 49 % males	29 weeks	9 weeks	(1) Placebo Human milk fortified with 0.5ml of soy oil and medium-chain triglyceride per 100 ml (2) Human milk fortified with 0.5 ml of soy oil per 100 ml (32 mg of DHA and 31 mg of AA)	Yes	HM or DHM	Yes	Cognitive and neurodevelopmental outcomes Full scale IQ (FSIQ) Growth and anthropometry outcomes Anthropometric data	No significant differences in weight-for-age or length-for-age z-scores between groups at any time point. IGF-1 levels were significantly lower in group 2 at 8 years. DHA concentration at follow-up was a significant predictor of higher IQ at 8 years, suggesting a potential long-term impact of group 2 on cognitive development.	Low risk ¹
Wendel et al. ⁽⁴⁰⁾ 2023, Norway	RCT	n = 120 40 weeks 55 % males	26,4 weeks	36 weeks (starting 2 days after birth)	(1) Placebo Human milk with MCT-oil (0.4 ml/kg) (2) Human milk fortified with AA:DHA ratio 2:1 (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM or DHM	Yes	Respiratory outcomes Bronchopulmonary dysplasia (BPD)	Group 2 resulted in improved respiratory outcomes with fewer days of respiratory support and a lower oxygen demand (FiO ₂) compared to group 1. No differences observed in the incidence of BPD and need for respiratory support at 40 weeks.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration of supplementation (initial age)	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Rossholt et al. ⁽³⁹⁾ 2023, Norway	RCT	n = 97 3 months 57,7 % males	26,5 weeks	36 weeks (starting 2 days after birth)	(1) Placebo Human milk with MCT-oil (0.4 ml/kg) (2) Human milk fortified with AA:DHA 2:1 (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM or DHM	Yes	Growth and anthropometry outcomes Anthropometric data	Group 2 exhibited better linear growth from birth to term-equivalent age. There was no sustained effect on growth at 3 months. There were no significant differences in body composition between the groups. Breastfeeding supplemented with AA:DHA (group 2) was positively associated with fat-free mass z-score and negatively associated with fat mass z-score.	Low risk ¹
Alshweki et al. ⁽⁵⁴⁾ 2015, Spain	RCT	n = 71 2 years 49,3 % males	30 weeks	14 months	(1) Human Milk (2) Placebo Human milk fortified with ω -6/ ω -3 ratio of 1/1 (3) Human milk fortified with ω -6/ ω -3 2/1	Yes	HM and DHM	No	Cognitive and neurodevelopmental outcomes Psychomotor development	Group 3 had higher blood levels of essential fatty acids during the first year of life, at 6 and 12 months. Group 3 also achieved a higher significantly average in Brunet Lézine score at 24 months comparing to group 2. Group 2 had worse results than group 1 in both proportion of children scoring below 85 and average scores.	Low risk ¹
Cortijo et al. ⁽⁵⁶⁾ 2020, Spain	RCT	n = 22 65 weeks 40,9 % males	28,9 weeks	36 weeks	(1) Placebo Human milk fortified with MCT-oil (2) Human milk fortified with fungi and microalgae oil with AA:DHA 2:1 (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM and DHM	Yes	Biomarkers and Nutritional Status Plasma oxidative status	Group 2 had altered plasma fatty acid profiles, lower lipid peroxidation levels (linoleic and dihomo- γ -linolenic), and higher antioxidant activity compared to group 1. However, there were no significant differences in neonatal growth outcomes between the two groups.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration of supplementation (initial age)	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Hellström et al. ⁽³⁴⁾ 2021, Sweden	RCT	n = 175 40 weeks 56,6% males	25 weeks	40 weeks (starting 3 days after birth)	(1) Placebo Human milk (2) Human milk fortified with fungi and microalgae oil with AA:DHA 2:1 (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM or DHM	Yes	Visual outcomes Retinopathy of Prematurity (ROP)	During the first 28 CA days, lower serum DHA levels, as group 1, was associated with severe ROP. Otherwise, AA levels showed no association with ROP. Lower levels of eicosadienoic acid during these days remained associated with severe ROP even after adjusting for GA and BW.	Low risk ¹
Hellström et al. ⁽³³⁾ 2021, Sweden	RCT	n = 206 40 weeks 56,8 % males	25,5 weeks	40 weeks (starting 3 days after birth)	(1) Placebo Human milk (2) Human milk fortified with fungi and microalgae oil with AA:DHA 2:1 (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM or DHM	Yes	Visual and Respiratory outcomes Retinopathy of Prematurity (ROP), Bronchopulmonary Dysplasia (BPD)	Group 2 had the incidence of severe ROP significantly reduced by 50% compared to group 1. There were no significant differences in morbidities such as BPD, necrotizing enterocolitis, PDA, IVH, or postnatal growth between the groups.	Low risk ¹
Wezel-Meijler et al. ⁽³⁶⁾ 2002, Sweden	Case-control study	n = 42 6 months 34,1 % males	20 weeks	6 months	(1) Placebo Preterm formula (2) Preterm formula with DHA:AA 2:1 At 3000g, infants were transitioned to infant formula accordingly.	Yes	Formula	Yes	Cognitive and neurodevelopmental and Visual outcomes Visual evoked potential (VEP), visual acuity	Group 2 showed no significant differences in cerebral myelination, flash-VEPs, or developmental outcomes. Visual acuity tended to be slightly higher in group 2, but the difference was not statistically significant.	5 ²
Lundgren et al. ⁽³⁵⁾ 2023, Sweden	RCT	n = 115 2,7 years 58,3 % males	25,1 weeks	40 weeks (starting 3 days after birth)	(1) Placebo Human milk (2) Human milk fortified with fungi and microalgae oil with AA:DHA (100 mg/kg of AA and 50 mg/kg of DHA)	Yes	HM	Yes	Visual outcomes Visual acuity	Group 2 had significantly higher visual acuity than group 1. Hyperopia was more common, while myopia occurred only in group 1. Strabismus rates were similar in both groups.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration of supplementation (initial age)	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
Ryan et al. ⁽⁴⁹⁾ 1999, United States	RCT	n = 63 59 weeks 50,8 % males	31 weeks	59 weeks	(1) Placebo Neonatal care (2) Neonatal care with DHA from fish oil with DHA:EPA 5:1 At 43 weeks, group 1 transitioned to a term formula and group 2 to a term formula with DHA.	No	HM or formula	No	Growth and anthropometry outcomes Anthropometric data	Among males in group 2 there was lower gains in weight, length, and head circumference. However, there was no significant differences in females between groups. Group 2 males had lower fat-free mass compared to group 1 at 51- and 59-weeks CA with no differences in females.	Low risk ¹
Frost et al. ⁽⁴⁵⁾ 2021, United States	RCT	n = 30 36 weeks 40,0 % males	28 weeks	8 weeks	(1) Placebo Sunflower oil (2) Neonatal care LCPUFA-120 [40 mg/kg/day DHA + 80 mg/kg/day AA] (3) Neonatal care LCPUFA-360 [120 mg/kg/day DHA + 240 mg/kg/day AA]	Yes	HM or DHM and formula	Yes	Biomarkers and Nutritional Status Plasma Composition	Group 3 showed the highest increase in DHA and AA levels compared to the groups 1 and 2. Blood DHA levels were significantly different between groups at 2 and 8 weeks, with group 3 showing the highest levels.	Low risk ¹
Robinson et al. ⁽²⁷⁾ 2016, United States	RCT	n = 28 8 years 60,7 % males	26 weeks	8 weeks (starting 3 days after birth)	(1) Placebo Preterm formula (2) Preterm formula with DHA (20 mg/kg/day) + AA (40 mg/kg/day) (3) Preterm formula with DHA (60 mg/kg/day) + AA (120 mg/kg/day)	Yes	Formula	Yes	Biomarkers and Nutritional Status Red blood cell (RBC),	Group 2 did not change RBC phospholipids LCPUFA levels at 2 and 8 weeks. Daily buccal DHA and AA supplements did not affect fatty acid levels in groups 2 and 3.	Low risk ¹
Boone et al. ⁽⁴²⁾ 2017, United States	RCT	n = 31 18-38 months 67,7% males	27 weeks	90 days	(1) Placebo Daily canola oil (2) Daily oral omega fatty acid (lemon-flavored fish and borage oil)	No	Formula	No	Cognitive and neurodevelopmental outcomes Behaviour acuity	After 90 days, parents of group 2 infants reported reduced sensory sensitivity behaviours. Group 2 had higher neurological thresholds and lower distractibility and hyperactivity compared to group 1.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
David G. et al. ⁽⁵⁰⁾ 1992, United States	Case-control study	n = 74 6 months % males	30,4 weeks	6 months	(1) Human milk (2) Placebo Preterm formula corn oil-based (3) Preterm formula soy oil-based (4) Preterm formula with linoleic acid and marine oils [like HM]	No	HM and formula	No	Visual outcomes Retinal development	At 36 weeks, group 2 showed significantly higher rod thresholds compared to group 1 and 4. At 57 weeks, significant differences were not observed among groups in term of rod thresholds. Additionally, there were no significant differences among the groups in terms of cone b-wave responses at either time.	6 ²
Hoffman et al. ⁽⁴⁷⁾ 1992, United States	Case-control study	n = 95 13 months	30,1 weeks	57 weeks PMA (starting 10 days after birth)	(1) Human milk (2) Placebo Preterm formula corn oil-based (3) Preterm formula soy oil-based (4) Preterm formula with linoleic acid and marine oils [like HM]	No	HM and formula	No	Biomarkers and Nutritional Status Plasma Composition	At 36 weeks, group 1 infants showed significantly higher DHA concentrations comparing with the other groups. At 57 weeks CA, differences in fatty acid composition persisted, with group 1 and 4 infants showing higher ω -3 to ω -6 fatty acid ratios compared to group 2 and 3.	4 ²
Groh-Wargo et al. ⁽⁴⁶⁾ 2005, United States	RCT	n = 57 12 months 54,4 % males	30 weeks	12 months (starting 3 days after birth)	(1) Placebo Neonatal care (2) Neonatal care with DHA + AA (egg derived triglyceride and fish oil) (3) Neonatal care with DHA + AA (fungal and fish oil) Until 40 weeks, 24 cal/oz of Similac Special Care [®] and from 40 weeks, 22 cal/oz of NeoSure [®] (without fish oil).	Yes	HM or formula	No	Growth and anthropometry outcomes Anthropometric data	Group 2 and 3 demonstrated an increase in lean body mass and a decrease in fat mass at 12 months of CA. There were no notable variations among the groups in terms of weight, length, or head circumference. Additionally, there were no significant differences observed between 35 weeks and 12 months in groups 2, 3 compared to group 1 regarding growth or bone mineralization.	Low risk ¹

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Table 2. (Continued)

Study	Study design	Population (sample size, outcome age, sex)	Gestational age	Duration and initial age of supplementation	Concentration and source supplementation	Arachidonic acid (AA)	Feeding type	Parenteral nutrition	Health outcomes	Health outcomes associated with LCPUFA supplementation	Quality assessment score
O'Connor et al. ⁽⁴⁸⁾ 2001, United States	RCT	n = 422 20 months 54,7 % males	29,7 weeks	12 months (starting 3 days after birth)	(1) Human milk (2) Placebo Preterm formula (3) Preterm formula with DHA + AA (egg derived triglyceride and fish oil) (4) Preterm formula with DHA + AA (fungal and fish oil)	Yes	HM and formula	No	Cognitive and neurodevelopmental and Visual outcomes Behaviour acuity, Visual acuity, Visual evoked potential (VEP)	At 6 months, infants in groups 3 and 4 had superior VEP responses, indicating better visual and cognitive development than group 1. Group 3 also had higher behavioural acuity scores at 4 months CA.	Some concerns ¹
Carlson et al. ⁽⁴³⁾ 1992, United States	Case-control study	n = 65 26 months 36,9 % males	26 weeks	79 weeks	(1) Placebo Preterm formula (2) Preterm formula with marine oil From 79 weeks, infants transitioned to a term formula.	No	Formula	No	Growth and anthropometry outcomes Anthropometric data	At 40 weeks, group 2 negatively affected first-year growth with significantly lower Z-scores for weight, length, and head circumference, comparing with group 1.	8 ²
Carlson et al. ⁽⁴⁴⁾ 1996, United States	Case-control study	n = 65 12 months	28,6 weeks	48 weeks (starting 3 to 5 days after birth)	(1) Placebo Preterm formula (2) Preterm formula with marine oil After 2 months, infants transitioned to a term formula.	No	Formula	No	Respiratory, Visual, and Growth and anthropometry outcomes Bronchopulmonary dysplasia (BPD), Anthropometric data, Visual acuity	Fewer infants in group 2 developed BPD compared to group 1. Group 2 infants without BPD had better visual acuity at 2 months, but by 4 months, both groups showed poorer acuity regardless of diet. Additionally, group 2 infants weighed less at 6 and 9 months and had a lower weight-for-length ratio at 2, 6, 9, and 12 months.	4 ²

AA: arachidonic acid; BPD: bronchopulmonary dysplasia; DHA: docosahexaenoic acid; DHM: donor human milk; FSIQ: full-scale intelligence quotient; HM: human milk; NEC: necrotizing enterocolitis; LCPUFA: long-chain polyunsaturated fatty acids; PHL FA: phospholipid fatty acids; RCT: randomized controlled trial; ROP: retinopathy of prematurity; VLBW: very low birth weight; PPHM: pasteurized-pooled human milk.

¹ Tool of the Cochrane Collaboration for randomized control trials (low, medium, or high risk of bias).

² The NOS for case-control studies (varying from 0 to 9 stars).

6. References

1. World Health Organization. Preterm birth. 2023. Available at: <https://www.who.int/news-room/fact-sheets/detail/preterm-birth>. Accessed at may 2024.
2. Ohuma EO, Moller AB, Bradley E, Chakwera S, Hussain-Alkhateeb L, Lewin A, et al. National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet*. 2023; 402(10409):1261-71. [https://doi.org/10.1016/S0140-6736\(23\)00878-4](https://doi.org/10.1016/S0140-6736(23)00878-4).
3. Walani SR. Global burden of preterm birth. *Int J Gynaecol Obstet*. 2020; 150(1):31-33. <https://doi.org/10.1002/ijgo.13195>.
4. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. *Lancet*. 2008; 371(9606):75-84. [https://doi.org/10.1016/S0140-6736\(08\)60074-4](https://doi.org/10.1016/S0140-6736(08)60074-4).
5. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr*. 1994; 60(2):189-94. <https://doi.org/10.1093/ajcn/60.2.189>.
6. Peterson BS, Vohr B, Kane MJ, Whalen DH, Schneider KC, Katz KH, et al. A functional magnetic resonance imaging study of language processing and its cognitive correlates in prematurely born children. *Pediatrics*. 2002; 110(6):1153-62. <https://doi.org/10.1542/peds.110.6.1153>.
7. Smithers LG, Gibson RA, McPhee A, Makrides M. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. *Am J Clin Nutr*. 2008; 87(4):912-20. <https://doi.org/10.1093/ajcn/87.4.912>.

8. Lapillonne A, Moltu SJ. Long-Chain Polyunsaturated Fatty Acids and Clinical Outcomes of Preterm Infants. *Ann Nutr Metab.* 2016; 69 Suppl 1:35-44. <https://doi.org/10.1159/000448265>.
9. Silveira RC, Corso AL, Procianoy RS. The Influence of Early Nutrition on Neurodevelopmental Outcomes in Preterm Infants. *Nutrients.* 2023; 15(21) <https://doi.org/10.3390/nu15214644>.
10. Herrera E. Implications of Dietary Fatty Acids During Pregnancy on Placental, Fetal and Postnatal Development—A Review. *Placenta.* 2002; 23:S9-S19. <https://doi.org/10.1053/plac.2002.0771>.
11. Fleith M, Clandinin MT. Dietary PUFA for preterm and term infants: review of clinical studies. *Crit Rev Food Sci Nutr.* 2005; 45(3):205-29. <https://doi.org/10.1080/10408690590956378>.
12. Heath RJ, Klevebro S, Wood TR. Maternal and Neonatal Polyunsaturated Fatty Acid Intake and Risk of Neurodevelopmental Impairment in Premature Infants. *Int J Mol Sci.* 2022; 23(2). <https://doi.org/10.3390/ijms23020700>.
13. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr.* 1992; 120(4 Pt 2):S129-38. [https://doi.org/10.1016/S0022-3476\(05\)81247-8](https://doi.org/10.1016/S0022-3476(05)81247-8).
14. Alshaikh BN, Reyes Loredó A, Yusuf K, Maarouf A, Fenton TR, Momin S. Enteral long-chain polyunsaturated fatty acids and necrotizing enterocolitis: A systematic review and meta-analysis. *Am J Clin Nutr.* 2023; 117(5):918-29. <https://doi.org/10.1016/j.ajcnut.2023.01.007>.
15. Diggikar S, Aradhya AS, Swamy RS, Namachivayam A, Chandrasekaran M. Effect of Enteral Long-Chain Polyunsaturated Fatty Acids on Retinopathy of Prematurity: A Systematic Review and Meta-Analysis. *Neonatology.* 2022; 119(5):547-57. <https://doi.org/10.1159/000525266>.

16. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gøtzsche PC, Ioannidis JPA, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*. 2009; 339:b2700. <https://doi.org/10.1016/j.jclinepi.2009.06.006>.
17. Shamseer L, Moher D, Clarke M, Ghersi D, Alessandro Liberati, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. *BMJ : British Medical Journal*. 2015; 349:g7647. <https://doi.org/10.1136/bmj.g7647>.
18. Inês Agostinho, Beatriz Teixeira, Maria Manuela Cardoso. A systematic review about long-chain fatty acid supplementation in very preterm newborns: outcomes and tolerance. PROSPERO (CRD42023461646). 2023
19. Kohl CM, Emma J.; Unger, Stefan; Haddaway, Neal R.; Kecke, Steffen; Schiemann, Joachim; Wilhelm, Ralf Online tools supporting the conduct and reporting of systematic reviews and systematic maps: a case study on CADIMA and review of existing tools. *Environmental Evidence*. 2018. <https://doi.org/10.1186/s13750-018-0115-5>.
20. Harris JD, Quatman CE, Manring MM, Siston RA, Flanigan DC. How to write a systematic review. *The American journal of sports medicine*. 2014; 42(11):2761-68. <https://doi.org/10.1177/0363546513497567>.
21. Guidelines for snowballing in systematic literature studies and a replication in software engineering. *Proceedings of the 18th international conference on evaluation and assessment in software engineering*; 2014. <https://doi.org/10.1145/2601248.2601268>.
22. Sterne JA, Savović J, Page MJ, Elbers RG, Blencowe NS, Boutron I, et al. RoB 2: a revised tool for assessing risk of bias in randomised trials. *bmj*. 2019; 366. <https://doi.org/10.1136/bmj.l4898>.

23. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2000. Available at: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed at may 2024.
24. Bernabe-García M, Calder PC, Villegas-Silva R, Rodríguez-Cruz M, Chávez-Sánchez L, Cruz-Reynoso L, et al. Efficacy of Docosahexaenoic Acid for the Prevention of Necrotizing Enterocolitis in Preterm Infants: A Randomized Clinical Trial. *Nutrients*. 2021; 13(2). <https://doi.org/10.3390/nu13020648>.
25. Bernabe-García M, Villegas-Silva R, Villavicencio-Torres A, Calder PC, Rodríguez-Cruz M, Maldonado-Hernández J, et al. Enteral Docosahexaenoic Acid and Retinopathy of Prematurity: A Randomized Clinical Trial. *JPEN J Parenter Enteral Nutr*. 2019; 43(7):874-82. <https://doi.org/10.1002/jpen.1497>.
26. Almaas AN, Tamnes CK, Nakstad B, Henriksen C, Grydeland H, Walhovd KB, et al. Diffusion tensor imaging and behavior in premature infants at 8 years of age, a randomized controlled trial with long-chain polyunsaturated fatty acids. *Early Hum Dev*. 2016; 95:41-6. <https://doi.org/10.1016/j.earlhumdev.2016.01.021>.
27. Henriksen C, Almaas AN, Westerberg AC, Drevon CA, Iversen PO, Nakstad B. Growth, metabolic markers, and cognition in 8-year old children born prematurely, follow-up of a randomized controlled trial with essential fatty acids. *Eur J Pediatr*. 2016; 175(9):1165-74. <https://doi.org/10.1007/s00431-016-2755-1>.
28. Robinson DT, Caplan M, Carlson SE, Yoder R, Murthy K, Frost B. Early docosahexaenoic and arachidonic acid supplementation in extremely-low-birth-weight infants. *Pediatr Res*. 2016; 80(4):505-10. <https://doi.org/10.1038/pr.2016.118>.

29. Collins CT, Makrides M, McPhee AJ, Sullivan TR, Davis PG, Thio M, et al. Docosahexaenoic Acid and Bronchopulmonary Dysplasia in Preterm Infants. *N Engl J Med*. 2017; 376(13):1245-55. . <https://doi.org/10.1056/NEJMoa1611942>.
30. Gould JF, Makrides M, Gibson RA, Sullivan TR, McPhee AJ, Anderson PJ, et al. Neonatal Docosahexaenoic Acid in Preterm Infants and Intelligence at 5 Years. *N Engl J Med*. 2022; 387(17):1579-88. <https://doi.org/10.1056/NEJMoa2206868>.
31. Collins CT, Gibson RA, Anderson PJ, McPhee AJ, Sullivan TR, Gould JF, et al. Neurodevelopmental outcomes at 7 years' corrected age in preterm infants who were fed high-dose docosahexaenoic acid to term equivalent: a follow-up of a randomised controlled trial. *BMJ Open*. 2015; 5(3):e007314. <https://doi.org/10.1136/bmjopen-2014-007314>.
32. Tanaka K, Hosozawa M, Kudo N, Yoshikawa N, Hisata K, Shoji H, et al. The pilot study: sphingomyelin-fortified milk has a positive association with the neurobehavioural development of very low birth weight infants during infancy, randomized control trial. *Brain Dev*. 2013; 35(1):45-52. <https://doi.org/10.1016/j.braindev.2012.03.004>.
33. Abou El Fadl DK, Ahmed MA, Aly YA, Darweesh EAG, Sabri NA. Impact of Docosahexaenoic acid supplementation on proinflammatory cytokines release and the development of Necrotizing enterocolitis in preterm Neonates: A randomized controlled study. *Saudi Pharm J*. 2021; 29(11):1314-22. <https://doi.org/10.1016/j.jsps.2021.09.012>.
34. Hellström A, Nilsson AK, Wackernagel D, Pivodic A, Vanpee M, Sjöbom U, et al. Effect of Enteral Lipid Supplement on Severe Retinopathy of Prematurity: A Randomized Clinical Trial. *JAMA Pediatr*. 2021; 175(4):359-67. <https://doi.org/10.1001/jamapediatrics.2020.5653>.
35. Hellström A, Pivodic A, Gränse L, Lundgren P, Sjöbom U, Nilsson AK, et al. Association of Docosahexaenoic Acid and Arachidonic Acid Serum Levels With

- Retinopathy of Prematurity in Preterm Infants. *JAMA Netw Open*. 2021; 4(10):e2128771. <https://doi.org/10.1001/jamanetworkopen.2021.28771>.
36. Lundgren P, Jacobson L, Gränse L, Hård AL, Sävman K, Hansen-Pupp I, et al. Visual outcome at 2.5 years of age in ω -3 and ω -6 long-chain polyunsaturated fatty acid supplemented preterm infants: a follow-up of a randomized controlled trial. *Lancet Reg Health Eur*. 2023; 32:100696. <https://doi.org/10.1016/j.lanepe.2023.100696>.
 37. van Wezel-Meijler G, van der Knaap MS, Huisman J, Jonkman EJ, Valk J, Lafeber HN. Dietary supplementation of long-chain polyunsaturated fatty acids in preterm infants: effects on cerebral maturation. *Acta Paediatr*. 2002; 91(9):942-50. <https://doi.org/10.1111/j.1651-2227.2002.tb02882.x>.
 38. Henriksen C, Haugholt K, Lindgren M, Aurvåg AK, Rønnestad A, Grønn M, et al. Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. *Pediatrics*. 2008; 121(6):1137-45. <https://doi.org/10.1542/peds.2007-1511>.
 39. Moltu SJ, Blakstad EW, Strømmen K, Almaas AN, Nakstad B, Rønnestad A, et al. Enhanced feeding and diminished postnatal growth failure in very-low-birth-weight infants. *J Pediatr Gastroenterol Nutr*. 2014; 58(3):344-51. <https://doi.org/10.1097/MPG.0000000000000220>.
 40. Rossholt ME, Bratlie M, Wendel K, Aas MF, Gunnarsdottir G, Fugelseth D, et al. Effect of arachidonic and docosahexaenoic acid supplementation on quality of growth in preterm infants: A secondary analysis of a randomized controlled trial. *Clin Nutr*. 2023; 42(12):2311-19. <https://doi.org/10.1016/j.clnu.2022.11.012>.
 41. Wendel K, Aas MF, Gunnarsdottir G, Rossholt ME, Bratlie M, Nordvik T, et al. Effect of arachidonic and docosahexaenoic acid supplementation on respiratory outcomes and neonatal morbidities in preterm infants. *Clin Nutr*. 2023; 42(1):22-28. <https://doi.org/10.1016/j.clnu.2023.10.005>.

42. Westerberg AC, Schei R, Henriksen C, Smith L, Veierød MB, Drevon CA, et al. Attention among very low birth weight infants following early supplementation with docosahexaenoic and arachidonic acid. *Acta Paediatr.* 2011; 100(1):47-52. <https://doi.org/10.1111/j.1651-2227.2010.01946.x>.
43. Boone KM, Gracious B, Klebanoff MA, Rogers LK, Rausch J, Coury DL, et al. Omega-3 and -6 fatty acid supplementation and sensory processing in toddlers with ASD symptomology born preterm: A randomized controlled trial. *Early Hum Dev.* 2017; 115:64-70. <https://doi.org/10.1016/j.earlhumdev.2017.09.015>.
44. Carlson SE, Cooke RJ, Werkman SH, Tolley EA. First year growth of preterm infants fed standard compared to marine oil n-3 supplemented formula. *Lipids.* 1992; 27(11):901-7. <https://doi.org/10.1007/BF02535870>.
45. Carlson SE, Werkman SH, Tolley EA. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. *Am J Clin Nutr.* 1996; 63(5):687-97. <https://doi.org/10.1093/ajcn/63.5.687>.
46. Frost BL, Patel AL, Robinson DT, Berseth CL, Cooper T, Caplan M. Randomized Controlled Trial of Early Docosahexaenoic Acid and Arachidonic Acid Enteral Supplementation in Very Low Birth Weight Infants. *J Pediatr.* 2021; 232:23-30.e1. <https://doi.org/10.1016/j.jpeds.2020.12.037>.
47. Groh-Wargo S, Jacobs J, Auestad N, O'Connor DL, Moore JJ, Lerner E. Body composition in preterm infants who are fed long-chain polyunsaturated fatty acids: a prospective, randomized, controlled trial. *Pediatr Res.* 2005; 57(5 Pt 1):712-8. <https://doi.org/10.1203/01.pdr.0000156509.29310.55>.
48. Hoffman DR, Uauy R. Essentiality of dietary omega 3 fatty acids for premature infants: plasma and red blood cell fatty acid composition. *Lipids.* 1992; 27(11):886-95. <https://doi.org/10.1007/BF02535868>.

49. O'Connor DL, Hall R, Adamkin D, Auestad N, Castillo M, Connor WE, et al. Growth and development in preterm infants fed long-chain polyunsaturated fatty acids: a prospective, randomized controlled trial. *Pediatrics*. 2001; 108(2):359-71. <https://doi.org/10.1542/peds.108.2.359>.
50. Ryan AS, Montalto MB, Groh-Wargo S, Mimouni F, Sentipal-Walerius J, Doyle J, et al. Effect of DHA-containing formula on growth of preterm infants to 59 weeks postmenstrual age. *Am J Hum Biol*. 1999; 11(4):457-67. [https://doi.org/10.1002/\(SICI\)1520-6300\(1999\)11:4%3C457::AID-AJHB5%3E3.0.CO;2-B](https://doi.org/10.1002/(SICI)1520-6300(1999)11:4%3C457::AID-AJHB5%3E3.0.CO;2-B).
51. Birch DG, Birch EE, Hoffman DR, Uauy RD. Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci*. 1992; 33(8):2365-76.
52. Lapillonne A, Picaud JC, Chirouze V, Goudable J, Reygrobellet B, Claris O, et al. The use of low-EPA fish oil for long-chain polyunsaturated fatty acid supplementation of preterm infants. *Pediatr Res*. 2000; 48(6):835-41. <https://doi.org/10.1203/00006450-200012000-00022>.
53. Vanderhoof J, Gross S, Hegyi T. A multicenter long-term safety and efficacy trial of preterm formula supplemented with long-chain polyunsaturated fatty acids. *J Pediatr Gastroenterol Nutr*. 2000; 31(2):121-7. <https://doi.org/10.1097/00005176-200008000-00007>.
54. Bernhard W, Böckmann K, Maas C, Mathes M, Hövelmann J, Shunova A, et al. Combined choline and DHA supplementation: a randomized controlled trial. *Eur J Nutr*. 2020; 59(2):729-39. <https://doi.org/10.1007/s00394-019-01940-7>.
55. Alshweki A, Muñuzuri AP, Baña AM, de Castro MJ, Andrade F, Aldamiz-Echevarría L, et al. Effects of different arachidonic acid supplementation on psychomotor development in very preterm infants; a randomized controlled trial. *Nutr J*. 2015; 14:101. <https://doi.org/10.1186/s12937-015-0091-3>.

56. Martinez FE, Sieber VM, Jorge SM, Ferlin ML, Mussi-Pinhata MM. Effect of supplementation of preterm formula with long chain polyunsaturated Fatty acids on mineral balance in preterm infants. *J Pediatr Gastroenterol Nutr.* 2002; 35(4):503-7. <https://doi.org/10.1097/00005176-200210000-00008>.
57. Ramiro-Cortijo D, López de Pablo Á L, López-Giménez MR, Martin CR, Brown J, de Pipaón MS, et al. Plasma Oxidative Status in Preterm Infants Receiving LCPUFA Supplementation: A Pilot Study. *Nutrients.* 2020; 12(1). <https://doi.org/10.3390%2Fnu12010122>.
58. Billeaud C, Boué-Vaysse C, Couëdelo L, Steenhout P, Jaeger J, Cruz-Hernandez C, et al. Effects on Fatty Acid Metabolism of a New Powdered Human Milk Fortifier Containing Medium-Chain Triacylglycerols and Docosahexaenoic Acid in Preterm Infants. *Nutrients.* 2018; 10(6). <https://doi.org/10.3390%2Fnu10060690>.
59. Kaempf-Rotzoll DE, Hellstern G, Linderkamp O. Influence of long-chain polyunsaturated fatty acid formula feeds on vitamin E status in preterm infants. *Int J Vitam Nutr Res.* 2003; 73(5):377-87. <https://doi.org/10.1024/0300-9831.73.5.377>.
60. Agostoni C, Buonocore G, Carnielli VP, De Curtis M, Darmaun D, Decsi T, et al. Enteral nutrient supply for preterm infants: commentary from the European Society of Paediatric Gastroenterology, Hepatology and Nutrition Committee on Nutrition. *J Pediatr Gastroenterol Nutr.* 2010; 50(1):85-91. <https://doi.org/10.1097/mpg.0b013e3181adaee0>.
61. Suganuma M, Rumbold AR, Miller J, Chong YF, Collins CT. A Systematic Review and Meta-Analysis of Human Milk Feeding and Short-Term Growth in Preterm and Very Low Birth Weight Infants. *Nutrients.* 2021; 13(6). <https://doi.org/10.3390/nu13062089>.
62. Ginovart G, Gich I, Verd S. Human milk feeding protects very low-birth-weight infants from retinopathy of prematurity: a pre-post cohort analysis. *The journal of maternal-fetal & neonatal medicine: the official journal of the European Association of*

- Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstetricians. 2016; 29. <https://doi.org/10.3109/14767058.2016.1145648>.
63. Andreas NJ, Kampmann B, Mehring Le-Doare K. Human breast milk: A review on its composition and bioactivity. *Early Hum Dev.* 2015; 91(11):629-35. <https://doi.org/10.1016/j.earlhumdev.2015.08.013>.
64. Mihatsch W, Shamir R, van Goudoever JB, Fewtrell M, Lapillonne A, Lohner S, et al. ESPGHAN/ESPEN/ESPR/CSPEN guidelines on pediatric parenteral nutrition: Guideline development process for the updated guidelines. *Clin Nutr.* 2018; 37(6 Pt B):2306-08. <https://doi.org/10.1016/j.clnu.2018.06.943>.
65. Liu MY, Chen YY, Hu SH, Chen YK, Chang SJ. The Influence of Aggressive Parenteral Nutrition to Preterm and Very Low Birth Weight Infants. *Glob Pediatr Health.* 2015; 2:2333794x14567192. <https://doi.org/10.1177%2F2333794X14567192>.
66. Salas Lorenzo I, Chisaguano Tonato AM, de la Garza Puentes A, Nieto A, Herrmann F, Dieguez E, et al. The Effect of an Infant Formula Supplemented with AA and DHA on Fatty Acid Levels of Infants with Different FADS Genotypes: The COGNIS Study. *Nutrients.* 2019; 11(3). <https://doi.org/10.3390%2Fnu11030602>.
67. Jasani B, Simmer K, Patole SK, Rao SC. Long chain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev.* 2017; 3(3):Cd000376. <https://doi.org/10.1002/14651858.CD000376.pub4>.
68. Marc I, Boutin A, Pronovost E, Perez Herrera NM, Guillot M, Bergeron F, et al. Association Between Enteral Supplementation With High-Dose Docosahexaenoic Acid and Risk of Bronchopulmonary Dysplasia in Preterm Infants: A Systematic Review and Meta-analysis. *JAMA Netw Open.* 2023; 6(3):e233934. <https://doi.org/10.1001/jamanetworkopen.2023.3934>.

69. Wang Q, Zhou B, Cui Q, Chen C. Omega-3 Long-chain Polyunsaturated Fatty Acids for Bronchopulmonary Dysplasia: A Meta-analysis. *Pediatrics*. 2019; 144(1). <https://doi.org/10.1542/peds.2019-0181>.
70. Carlson S, Werkman S, Peeples J, Cooke R, Tolley EA. Carlson, S.E., Werkman, S.H., Peeples, J.M., Cooke, R.J. & Tolley, E.A. Arachidonic acid status correlates with the first year growth in preterm infants. *Proc. Natl. Acad. Sci. USA* 90, 1073-1077. *Proceedings of the National Academy of Sciences of the United States of America*. 1993; 90:1073-7. <https://doi.org/10.1073/pnas.90.3.1073>.
71. Angoa G, Pronovost E, Ndiaye ABKT, Lavoie PM, Lemyre B, Mohamed I, et al. Effect of Maternal Docosahexaenoic Acid Supplementation on Very Preterm Infant Growth: Secondary Outcome of a Randomized Clinical Trial. *Neonatology*. 2022; 119(3):377-85. <https://doi.org/10.1001/jama.2020.8896>.

**A systematic review about long-chain fatty
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