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Omega 3 fatty acids and inborn errors of metabolism

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Abstract

A number of studies are investigating the role of *n*-3 polyunsaturated fatty acids in children with metabolic inborn errors, while the effects on visual and brain development in premature infants and neonates are well known. However, their function incertain chronic neurological, inflammatory and metabolic disorders is still under study. Standards should be established to help identify the need of docosahexaenoic acid supplementation in conditions requiring a restricted diet resulting in an altered metabolism system, and find scientific evidence on the effects of such supplementation. This study reviews relevant published literature to propose adequate *n*-3 intake or supplementation doses for different ages and pathologies. The aim of this review is to examine the effects of long chain polyunsaturated fatty acids supplementation in preventing cognitive impairment or in retarding its progress, and to identify nutritional deficiencies, in children with inborn errors of metabolism. Trials were identified from a search of the Cochrane and MEDLINE databases in 2011. These databases include all major completed and ongoing double-blind, placebo-controlled, randomized trials, as well as all studies in which omega-3 supplementation was administered to children with inborn errors, and studies assessing omega-3 fatty acids status in plasma in these pathologies. Although few randomized controlled trials met the inclusion criteria of this review, some evidenced that most of children with inborn errors are deficient in omega-3 fatty acids, and demonstrated that supplementation might improve their neural function, or prevent the progression of neurological impairment. Nontheless, further investigations are needed on this issue.

Key words: docosahexaenoic: amino acid metabolism: peroxisomal disorders: urea cycle disorders

Background

Description of the condition

The brain, retina and other nervous tissues are particularly rich in long chain polyunsaturated fatty acids (LCPUFA). Many clinical and epidemiological studies have proven *n*-3 LCPUFA to produce positive effects on neural development in childhood[1–6].

In addition to their structural role, these fatty acids have effects that counteracting inflammation, platelet aggregation, hypertension and hyperlipidemia. These beneficial effects may be mediated by different mechanisms, including alterations in cell membrane composition and function, gene expression or as precursors of eicosanoid production[7]. Scientists have recently recommended to increase the intake of *n*-3 fatty acids, specifically docosahexaenoic acid (DHA), in children with chronic neurologic/metabolic diseases where dietary restrictions may limit *n*-3 LCPUFA intakes and cause a deficiency[8].

LCPUFA Physiology

Docosahexaenoic acid (DHA, 22:6*n*-3) and arachidonic acid (AA, 20:4*n*-6) are the quantitatively the most important LCPUFA of the *n*-3 and *n*-6 series respectively. Delta-6 desaturation of *n*-3 and *n*-6 precursor essential fatty acids converts alpha-linolenic acid and linoleic acid to intermediates that are elongated to C20 products and further desaturated via delta-5-desaturase, which yields eicosapentaenoic acid (EPA, C20:5*n*-3) and AA. Although AA is the major conversion product of the *n*-6 family, EPA serves as an intermediate which is further elongated and converted by delta-6 desaturation to C:24:6 *n*-3 which, after partial beta oxidation, yields DHA. The rate of endogenous essential fatty acid conversion by this desaturating enzyme is low and does not suffice to establish similar LCPUFA levels in subjects devoid of exogenous LCPUFA supply as compared with subjects with a habitual intake of a LCPUFA containing diet[9].

DHA is found in small amounts in most tissues, it is a major component of brain structure as part of cell membranes, with roles in signalling and neuronal growth[10]. DHA biosynthesis requires the elongation/desaturation enzymes that are shared by the *n*-3 and *n*-6 pathways[11]. Thus, DHA synthesis may be limited despite abundance of ALA and EPA[12]. Low DHA affects normal neurogenesis, neuronal and retinal signaling pathways[10]. During the postnatal period, poor neural connections are created if DHA supply is low, and maturation of visual

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cortical function as measured by acuity development will be slower[13]. DHA is a precursor of docosanoids, such as neuroprotectin D1, which inhibits oxidative stress and promotes cell survival. The new molecular functions of DHA, recently described, suggest their potential role in the development of therapies for neural and retinal degenerative diseases[14].

DHA requirements and recommended intake

DHA is not considered an essential fatty acid because it can be synthesized from ALA. Given the limited and high variability in the formation of DHA from ALA (1–5%), and because of its critical functions in visual and neurological development and other systems, especially in situations of possible nutritional deficiency, this fatty acid should be considered conditionally essential.[11] Endogenous synthesis contributes in a limited manner to plasma DHA levels, DHA is primarily provided by diet (fatty fish, for example). Indeed, the significance of preformed DHA intake from foods or supplements has been clearly demonstrated in numerous studies showing a marked increase in DHA blood levels[9,1516].

The average dietary intake of preformed AA and DHA in European adults consuming omnivorous diets is estimated at about 200 mg/day of each AA and DHA. However, the minimum requirements of DHA and recommended dietary intakes (DRI) in childhood are still uncertain[17]. In addition to being synthesized from its precursors, it is unclear when plasma DHA concentrations can be considered deficient. Moreover, no clinical or laboratory test has ever determined what can be considered DHA deficiency, and the time required to observe the conclusive effects of DHA supplementation has not yet been determined[18].

Taking into account that scientific knowledge is still limited, dietary recommendations should be individualized according to specific diseases. In children with chronic diseases and at risk of deficiency, supplementation or fortification of the dietary intake must be evaluated. Normally, the dietary supply of *n*-3 fatty acids in infancy depends on the duration of breastfeeding, the intake of fish that increases with age after weaning[19,20]. In the past, the use of different types of oils, marine foods and other supplements containing LCPUFA provided possibilities for dietary supplementation. Regular dietary intakes of oily fish provides preformed *n*-3 LCPUFA such as DHA and EPA. The current strategy to improve LCPUFA status is providing these acids through infant formula. Pure DHA extracted from fish oil, egg or single cell organisms (microalgae) is included in the formulas or feeding modules[21]. To secure bioavailability, it may also be necessary to evaluate the biochemical responses and metabolic and functional effects of formulas supplemented with DHA or LCPUFA.

Why is it important to do this review: Health Effects

The essential role of fats in children was evidenced early in the 1960s in malnourished children given skimmed milk. Fat-free parenteral nutrition was used in the late 1960 and early 70s. These children had impaired growth as well as dryness, scaling and thickening of the skin as a manifestation of the deficiency. *N*-3 fatty acids deficiency also causes dermatological diseases but usually in a subclinical form often associated with a deficiency of *n*-6 and other micronutrients. In these patients, such deficiency had negative effects on their neural development, visual function and peripheral neuropathy, which led to the investigation of the effects of *n*-3 on health (Table 1). In some metabolic diseases, there is also a decrease in DHA affecting specific functions. Thus, it is necessary to include DHA as part of the nutritional supplementation to correct this deficiency; however, this situation also serves to assess the potential therapeutic effects of DHA for specific disease conditions. In certain metabolic diseases, nutrition is the only treatment available, and although it may not cure the condition it may provide a better quality of life for the patients affected[22].

Objectives

The aims of this systematic review were to update the reported roles and functions of omega-3 LCPUFA in children with selected inborn errors of metabolism, and to revise the effects of supplementation in preventing or retarding metabolic alterations. Finally, we are providing some recommendations for LCPUFA intake or supplementation in specific metabolic conditions.

Methods

Types of studies

The research question in this systematic review was *"Does omega-3 LC-PUFA affect metabolic functions in inborn errors?"* It included all randomized controlled trials (RCTs), double-blind or placebo-controlled trials, in humans with inborn errors in which omega-3 supplementation was compared with placebo. RCTs with prospective, parallel or crossover designs were all considered. Finally, studies in which

Table 1. Attributed effects of *n*-3 fatty acids on health

Physiological functions of *n*-3 fatty acids	Outcomes on health
Growth	Positive effects in preterms
Visual Function	Improvement of visual acuity in neonates
Cognitive Function	Improvement of motor functions and learning
Lipid profile	Reduction of hypertrygliceridemia
Blood pressure	Reduction of blood pressure
Inflammatory system	Anti-inflammatory effects
Inmmunology system	Prevention of allergy and autoinmmune diseases

the status of omega-3 fatty acids in plasma in children with inborn errors was assessed under non randomized conditions were also revised. The papers, or at least the abstract, had to be in English, but there was no restriction on publication type or sample size.

Criteria for considering studies for this review. Inclusion and exclusion criteria

For a study to be considered, dietary supplementation or a specific diet had to be administered, excluding those on dietary recommendations or self-reporting alone. Studies were also excluded if a supplement was administered that could potentially leads to confounding the effects of omega-3 fatty acids (FA) and if there was no ethical approval. Particularly, two studies in which effects of omega 3 fatty acids were published in little samples of children without other studies with similar results, were excluded.

Types of participants

Eligible participants were individuals of all ages with different rare inborn diseases of metabolism such as phenylketonuria, urea cycle disorders, peroxisomal disorders. There was no restriction on the basis of gender, ethnicity, study setting or other characteristics.

Types of interventions

LC-PUFA treatments were selected, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) or docosapentaenoic acid (DPA), either individually or in combination with each other or with another pharmacological treatment (or vitamin supplementation) if the study design allowed the effect of omega-3 FA to be isolated. There were no restrictions with regard to dosage or dose regimen.

Types of outcome measurements

These had to include changes in the following outcomes comparing measurements between baseline and the end of the intervention:

Primary outcomes
Cognitive measurements
Secondary outcomes
PUFA levels
Serum or plasma levels of aminoacids, organic acids
Neurological changes
Metabolic changes

Studies that evaluate other changes were excluded.

Main stages of the systematic review

Trials were identified by conducting a search through Cochrane and MEDLINE databases from January to March 2011. This register contained references from all major health care databases and some ongoing trial databases and other grey literature sources without restriction on publication date until the search date, using the following equations.

The following search MeSH terms were used:

Fatty acids, Unsaturated fatty acids, Fatty acids, omega 3, Fatty acids, essential, Fatty acids, non esterified, docosahexaenoic.

Amino Acid Metabolism, Phenylketonuria, Tyrosinemias, Propionic acidaemia, Acidemia, isovaleric, Methylmalonic acidaemia with homocystinuria, Glutaric aciduria.

Peroxisomal disorders.

Urea Cycle Disorders, Inborn, Ornithine Carbamoyltransferase Deficiency Disease, Carbamoyl Phosphate Synthetase 1 Deficiency, Hyperargininemia, Citrullinemias.

Aciduria, Argininosuccinic.

Mithocondrial fatty acid beta-oxidation multienzyme complex [Supplementary Concept].

The main equations used to identify the studies on the PubMed database were:

#1 "Fatty Acids"[MeSH] AND "Inborn Errors"[MeSH] AND "humans" "[MeSH], which yielded 1563 different articles. Next, the equation was modified to: #2 "Fatty Acids, Omega-3"[MeSH] AND "Inborn Errors "[MeSH]: 22 results. #3 "Fatty Acids, Omega-3"[MeSH] AND "Inborn Errors"[MeSH] AND "Randomized Controlled Trials" yielding only 2 results.

Working in this specific area, other MeSH terms were used in similar equations. Some examples are as follow:

#4 "Unsaturated Fatty Acids"[MeSH] AND "Phenylketonuria"[MeSH] yielded 45 articles. #5 "Unsaturated Fatty Acids"[MeSH] AND "Phenylketonuria"[MeSH] AND "docosahexaenoic acid" "[MeSH] yielded 21 articles.

In peroxisomal disorders: #6 "Unsaturated Fatty Acids"[MeSH] AND "Peroxisomal disorders"[MeSH] yielded 235 articles, and #6 "Unsaturated Fatty Acids"[MeSH] AND "Peroxisomal disorders"[MeSH] AND "docosahexaenoic acid" "[MeSH] yielded 48 articles.

A tool, known as SYSCOLLAB was developed and used for the selection, classification and validation of these articles.

To select the studies, abstracts of references obtained by the search were examined by the two reviewers (MGC and PSC) who eliminated all references evidently ineligible for inclusion. Each reviewer studied all of the remaining full texts independently and made a selection. In cases of disagreement, a consensus was reached on the final list after discussion among the reviewers.

Effects of the interventions

Children with chronic diseases such as inborn errors of metabolism (aminoacidopathies, disorders of the urea cycle, peroxisomal disorders) or cystic fibrosis, who frequently suffer dietary restrictions, or have food restrictions including foods rich in omega-3. In some cases, the metabolic disorder itself can contribute to the deficiency despite adequate nutritional therapy[22]. Deficiencies in omega-3, mainly DHA have been reported in association with phenylketonuria, peroxisomal disorders, with hyperactivity, attention deficiency disorders, and cystic fibrosis in children. In the adults, Alzheimer's disease and old age per se are associated with lower DHA levels[8].

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DHA has a special role in regulating blood pressure and it has antiarrhythmic properties as it increases the permeability of heart cell membranes. There are some studies that have also evaluated its impact on hemodinamic parameters in childhood[4]. The appropriate dose and treatment time for some inborn errors still needs to be determined. It is known that hyperhomocysteinemia in association with vitamin B12 deficiency, and increased platelet aggregation, probably due to dietary deficiency of *n*-3 fatty acids, constitute cardiovascular risk factors frequently observed in hyperhomocystinuria, but also in strict vegetarians[23].

Clinical trials with DHA supplementation in children with cystic fibrosis are underway to evaluate nutrition and growth, lung and pancreatic function, and the decrease in the production of inflammatory isoprostanes[22–25].

Effect of PUFAS on aminoacid metabolism disorders. Children with disorders of amino acid metabolism following strict diets with very low dietary protein intakes are commonly devoid of omega-3 sources. These children have a lower LCPUFA intake and depressed concentrations of DHA- but not of AA- in plasma and erythrocyte phospholipids. This suggests that endogenous arachidonic (AA) synthesis might guarantee an adequate AA status. However, the lower DHA status warrants further investigations regarding the impact of DHA supplementation on growth and development of these children[26].

Phenylketonuria. Phenylketonuria (PKU) is the most common autosomal recessive inborn error of amino acid metabolism in Europe. There is a congenital deficiency of phenylalanine (Phe) hydroxylase that impairs the oxidation of the amino acid phenylalanine to tyrosine. Children with PKU are treated with a low Phe diet in order to protect brain development from potential damage of high levels of Phe in blood. The resulting diet is deficient in whole animal foods, which are rich in Phe. Moreover, since animal foods are sources of LCPUFA, children with hyperphenylalaninemia (HPA) also show depressed levels of these molecules in circulating and erythrocyte lipids[1]. As LCPUFA may have a role in neurotransmission, their dietary lack could contribute to the suboptimal development of HPA patients[27].

DHA depletion has been demonstrated in PKU children and may account for subtle neurological deficiencies that are not explained by variations in plasma phenylalanine concentrations. DHA synthesis is also potentially impaired by the products of excess phenylalanine catabolism; this is proposed as an etiological factor in the neuropathy of this illness[9]. Endogenous conversion of ALA is not sufficient to provide adequate amounts of DHA to support optimal function, and hence DHA appears to be conditionally essential for children with PKU. Since brain development -especially the replacement of components such as DHA in the brain- continues beyond childhood, and it would be reasonable to think that these recommendations should be extended to the pre-adolescence, adolescence and adulthood[9,28].

The effects of LCPUFA supplementation on blood fatty acid status in PKU children have been assessed in double-blind placebo-controlled clinical trials. Some studies have examined possible improvements in fine motor and coordination skills[9,30] and in visual function and visual evoked potential some years after supplementation with DHA[27,29]. Results have indicated that preformed *n*-3 LC-PUFA are needed for neural normalcy in PKU children[31]. However, in studies that evaluate long-term effects of PUFA supplementation, improvements in DHA levels and visual evoked latencies at the end of supplementation returned to baseline after three years[27].

Although further studies are needed in these "high-risk" groups to establish appropriate doses, it seems appropriate to consider DHA as an essential fatty acid in both PKU-affected pregnant women and in patients who have low levels of these essential metabolites. Considering their nutritional characteristics, breast milk and breastfeeding should be given greater consideration as part of the nutritional therapy in patients with PKU and in those with other inborn errors of metabolism. Long-term dietary guidance adding long-chain polyunsaturated fatty acids, consideration of the neurotrophic and neuroprotective effects and monitoring of the nutritional status of patients with PKU should be part of a lifelong follow-up programme that continues for life. Current dietetic products for PKU infants without phenylalanine are usually enriched both with DHA and AA and have been shown to normalize blood LCPUFA levels compared to those typically found in fully breastfed infants. In most cases, LCPUFA supplementation is not routinely provided in PKU after infancy[32].

Organic acidaemias. In methylmalonic acidaemia and urea cycle disorders, which dietary treatment also implies a limitation in protein-rich foods a lower percentage of DHA and AA in plasma and erythrocyte phospholipids has also been reported. In these groups of metabolic patients, dietary supplementation is recommended, since this population is at high risk of abnormal psychomotor development[33].

Disturbances of fatty acid metabolism with accumulation of odd-chain fatty acids have been reported in propionic acidaemia (PA). PA is one of the most common inborn errors of organic acid metabolism. Treatment is based on a restricted dietary intake of propionic acid precursor amino acids and, hence, natural proteins, and as a consequence of this limited intake, the dietary intake of preformed LCPUFA is lower. Accumulated propionyl-CoA may also interfere with the conversion of the essential fatty acids linoleic acid and ALA to AA and DHA. It is not known whether the synthesis of LCPUFA is also affected although a study with 5 children with PA a decade ago, showed that in clinically stable children with PA, LCPUFA synthesis is not affected[34].

Branched-chain ketoacid dehydrogenase deficiency results in complex and volatile metabolic derangements that threaten brain development. Treatment for classical maple syrup urine disease (MSUD) should address the underlying physiology while also preventing specific nutritional deficiencies. Administration of some amino acids can optimize the competition with branched-chain amino acids (BCAAs) for entry into the brain and correct omega-3 deficiencies widespread among MSUD patients. Some diet formulas have been tried to evaluate the clinical course of patients, who often present decompensations. In a recent study using a formula with

ALA among other nutrients, increased omega-3 PUFA levels in red cell membrane on study formula were observed, however, DHA levels remained below the normal range[35].

Hyperhomocysteinemia and homocystinuria. Children with hyperhomocysteinemia and homocystinuria have increased platelet aggregation, possibly due to dietary lack of *n*-3 fatty acids; this situation constitutes a cardiovascular risk factor and is also frequently observed in vegetarians. In vegans, 700 mg/day of each EPA and DHA, and vitamin B12 increased incorporation of these fatty acids into plasma lipids in all of them, together with a significant reduction in maximum percentage or slope of platelet aggregation[23,36].

Effect of LCPUFAS on Peroxisomal Disorders. The beta-oxidation step in the pathway of PUFA biosynthesis requires a considerable exchange of unsaturated fatty acids between different subcellular compartments[37]. Several lines of evidence suggest that peroxisomes are the intracellular site of this beta-oxidation step.

Peroxisomal disorders are severe genetic disorders that involve the central nervous system, leading to severe psychomotor retardation, retinopathy, liver disease, and early death. In these disorders, peroxisomes are not normally formed and their enzymes are deficient. These disorders are characterized by biochemical abnormalities in which plasmalogen synthesis and beta-oxidation of very-long-chain fatty acids (VLCFA) are affected. A profound brain DHA deficiency has been observed, as well as low concentrations of this fatty acid in all tissues and the blood. Given the essential role of DHA in neuronal and retinal membranes, a DHA deficiency of this magnitude might be pathogenic and may be responsible for altered signal transduction associated with learning disabilities, cognitive and visual dysfunction. These children seem to have a metabolic defect in DHA biosynthesis, which has never been totally elucidated and treatment with DHA seems to improve metabolic and clinical items in these patients[38].

The site of the final step of DHA formation (the beta oxidation of C24:6 *n*-3) has not been fully elucidated. It has been suggested that peroxisomes, and not mitochondria, are involved in DHA formation by catalyzing the beta-oxidation of C24:6*n*-3 to C22:6*n*-3. Additional studies of fibroblasts from patients with X-linked adrenoleukodystrophy (X-ALD), straight-chain acyl-CoA oxidase (SCOX) deficiency, D-bifunctional protein (DBP) deficiency, and rhizomelic chondrodysplasia punctata type 1, and of fibroblasts from l-bifunctional protein and sterol carrier protein X (SCPx) knockout mice, have shown that the main enzymes involved in DHA biosynthesis are SCOX, DBP, and both 3-ketoacyl-CoA thiolase and SCPx. This study shows that C24:6*n*-3 is not an elongationproduct from C22:6*n*-3, but that C24:6*n*-3 is an intermediate in DHA synthesis[38].

Within the Zellweger syndrome spectrum, generalized peroxisomal disorders, -usually called peroxisomal biogenesis disorders- are lethal congenital diseases, where a number of biochemical reactions of lipid metabolism, which normally occur in the peroxisome, are affected. Among them, beta-oxidation of pristanic and VLCFA, as well as alpha-oxidation of phytanic acid, is defective. Biosynthesis of plasmalogens and biliary acids is also defective[38]. In patients with Zellweger syndrome (ZS), (a peroxisome biogenesis disorder characterized by the absence of functional peroxisomes), DHA levels are clearly lower especially in brain and retina but also in liver, kidney, and blood[39]. This might explain the blindness and generalized weakness of these children.

There are milder Zellweger variants, known as neonatal X-ALD and infantile Refsum's disease. However, these relatively milder phenotypes present most of the symptoms of classic ZS including severe mental retardation with myelination delay followed later by demyelination-, visual and hearing sensorineural defects and liver involvement. In X-ALD there is an accumulation of VLCFA caused by the impaired peroxisomal oxidation of these fatty acids. However, this is not caused by a deficiency in one of the enzymes of the oxidation system, but by a defect in the peroxisomal membrane protein ALDP (adrenoleukodystrophy protein). These results in impaired beta-oxidation of VLCFA including C26:0 and C24:0. Although DHA synthesis has been found to be normal in fibroblasts from an X-ALD patients, all patients presented the same clinical and biochemical abnormalities as those described for patients with a peroxisome biogenesis disorder, including deficient hexacosanoic acid (C26:0), pristanic acid oxidation and phytanic acid oxidation[39]. DHA supplementation would improve biochemical abnormalities, visual and neurological functions, and growth in affected individuals. In some studies[40–42], DHA supplementation normalized their blood values, increased plasmalogen concentrations increased in erythrocytes, reduced VLCFA in plasma, and liver enzymes almost returned to normal levels in most cases. Improvements in clinical aspects have also been referred, as in visual and neurological functions with an improvement in myelination, growth and liver function. However in a randomized double-blind placebo-controlled trial, DHA supplementation did not improve the visual function or growth of children with peroxisome assembly disorders, exception made of a patient with a single enzyme disorder in the peroxisomal beta-oxidation[43]. Although adequate supplementation was provided, the variety and severity of pre-existent deficiencies made this experience difficult to interpret. The limits of these studies are that there is no uniformity in the genetic etiology of the affected children and the restricted sample size and variability in age of participants may have missed a small treatment effect[43]. These results suggest a fundamental role of DHA in the pathogenesis of ZS and although further studies are required for long-term assessment, DHA therapy is strongly recommended, not only to alleviate symptoms in patients with life-threatening diseases, but also to clarify remaining questions regarding the role of DHA in health and disease[42] although this therapeutic use cannot be endorsed at this time[43].

Deficiency of Mitochondrial Fatty Acid Beta Oxidation and LCPUFA. In the deficiency of one of the following mitochondrial enzymes: carnitine palmitoyltransferase 1 (CPT1), carnitine acylcarnitine translocase (CACT), carnitinepalmitoyltransferase 2 (CPT2), and very long-chain acyl-CoA dehydrogenase (VLCAD), the first three enzymes are necessary for the transport of activated fatty acids across the inner mitochondrial membrane and the last enzyme is part of the mitochondrial-oxidation system. Peroxisome-deficient patients

Table 2. Summary of different studies in children with metabolic inborn errors and the effects of *n*-3 fatty acids

Clinical trials	Study	Disease	Supplement	Doses	Time	Outcomes
LaVoie *et al.* 2009[46]	Case-control descriptive study	21 PKU children (≤6y) and 23 control children				PKU had significantly lower concentrations in total erythrocyte lipid of the sum of the ω-3, ω-6, saturated and PUFA.
Koletzko *et al.* 2009[9]	Open clinical trial	PKU children (n: 38; 1–11y)	Fish oil supplement	Fish oil capsules (Ameu®, Omega Pharma, Berlin, Germany) providing 500 mg fish oil per capsule (35 % omega-3 fatty acids including 18 % EPA and 12 % DHA).	3 months	Faster visual evoked potential latencies, indicating more rapid central nervous system information processing. Improved outcomes in a test of motor function and coordination.
Koletzko *et al.* PLEFA 2009[31]	Open clinical trial	PKU children (n: 38; 1–11y)	Fish oil supplement	Fish oil capsules (Ameu®, Omega Pharma, Berlin, Germany) providing 500 mg fish oil per capsule (35 % omega-3 fatty acids including 18 % EPA and 12 % DHA).	3 months	Faster visual evoked potential latencies, indicating more rapid central nervous system information processing. Improved outcomes in a test of motor function and coordination.
Koletzko *et al.* 2007[19]	Double blind, randomized study	PKU (n:21 children; 1 month)	Aminoacid supplements with LCPUFAs (DHA and AA)	0·43 % AA and 0·23 % DHA	12 months	Enhances LCPUFA plasma status
Beblo *et al.* 2007[30]	Open clinical trial	PKU (n: 36 children; 1–11 years)	LCPUFA (DHA y EPA)	Fish oil capsules (Ameu®, Omega Pharma, Berlin, Germany) providing 500 mg fish oil per capsule (35 % omega-3 fatty acids including 18 % EPA and 12 % DHA). To ensure a dose of 15 mg DHA/kg body weight	90 days	Enhances *n*-3 LCPUFA levels and improves motor skills.
Agostoni *et al.* 2006[47]	Double blind, randomized study	PKU (n: 42 infants)	Infant formula with DHA and AA	DHA: 0·3 g/100 g fatty acids AA: 0·7 g/100 g fatty acids	12 months	Phenylalanine-free infant formula supplemented with LC-PUFA could minimize the risk of suboptimal status that infants with PKU.
Agostoni *et al.* 2003[27]	Double blind, placebo-controlled trial	HPA (n: 20 children)	DHA and EPA	0·3 % to 0·5 % of the daily energy requirements as LCPUFA	12 months Reevaluation after 3 y	No changes after 3y
Agostoni *et al.* 2000[29]	Double blind, placebo controlled trial	HPA (n:20 children)	DHA and EPA	0·3 % to 0·5 % of the daily energy requirements as LCPUFA	12 months	Increases of the DHA pool and improves visual functions.
Paker *et al.* 2010[44]	Double-blind, placebo-controlled randomized trial	50 children with peroxisomal disorders (1 months-10y)	Microencapsulated powder that contained DHA and AA from tryglicerides.	47 % DHA and 46 % AA: 100 mg/kg/d,	1 year follow-up period	No differences in the outcomes between the treated and untreated groups in biochemical function, electroretinogram, or growth.
Tanaka *et al.* 2007[41]		1 girl with Zellweger syndrome	Formula supplemented with MCT milk, DHA, Lorenzo's oil, and Lunaria oil.		2 weeks	Delay in neurological development. Liver dysfunction, and cholestasis improved after starting the dietary treatment. DHA increased and VLCFA levels decreased.
Martínez *et al.* 2000[43]	Open clinical trial	13 infants with peroxisomal disorders	DHA-EE	Vial: 50–500 mg DHA-EE in a total volume of 2 mL.	6 weeks and 7y	Plasmalogen concentrations of DHA increased in erythrocytes and, amounts of VLCFA decreased in plasma. Liver enzymes returned almost to normal. Most patients showed improvement in vision, liver function, muscle tone, and social contact. In some patients, improved of brain myelin was detected.

Arachidonic acid (AA), docosahexaenoic acid (DHA) docosahexaenoic acid ethyl ester (DHA-EE), eicosapentaenoic acid (EPA), hyperphenylalaninemia (HPA), long chain polyunsaturated fatty acids (LCPUFA), middle chain triglyceride (MCT), Phenylketonuria (PKU), very-long-chain fatty acids (VLCFA).

do not convert 24:6*n*-3 to 22:6*n*-3, whereas this conversion seems to be normal in fibroblasts from patients with a mitochondrial fatty acid oxidation defect[39]. This confirms that DHA biosynthesis in human fibroblasts is a peroxisome-dependent process[44].

Adverse effects of LCPUFA supplementation in metabolic inborn errors. DHA, being a highly unsaturated fatty acid, is very susceptible to lipid peroxidation. Therefore, for a safe use of LCPUFA supplemented formulas, appropriate antioxidant protection shoud be provided, as free radicals might contribute to neural tissue damage. However, several studies in children did not find any differences in native lipid peroxide concentrations or in antioxidant enzyme activity[45]. The optimal type and dose of supplement remain to be determined. As regards the adverse effects of DHA overdose, randomized studies administered 1 g/day of DHA or 2–7 g/day of *n*-3 LCPUFA and no adverse effects were reported, even during pregnancy[19]. One alternative to supplementation, particularly in children with chronic diseases in which the DHA may be essential, is the administration of modules or capsules of this fatty acid, where plasma levels should be monitored during the treatment. It is important to supply both *n*-3 and *n*-6 fatty acids to avoid biochemical imbalances. In some studies, patients received fatty acids as LCPUFA supplementation containing equivalent amounts of *n*-3 and *n*-6 fatty acids[29].

In paediatric studies fish oil has been commonly used as a supplement (15 mg DHA/kg body weight daily) for approximately 3 months; although it has been associated with a reduction in AA values, authors have suggested that the dose might well be higher than the dose needed for optimal outcomes[9,31]. Gelatin capsules with 500 mg of oil might also be supplied to ensure that 0·3 %-0·5 % of the daily energy requirements is met, following expert advice that specifies that 0·27 % of the daily energy requirements should be provided in the form of *n*-3 LCPUFA in healthy subjects[29].

Discussion and author's conclusions

Implications for practice

This review found that some children with inborn errors (HPA, PKU, or ZS) present omega-3 fatty acid deficiency and suggests that omega-3 PUFA supplementation over long periods might have positive effects, as it prevents the development of cognitive impairment in children, and inhibits the onset of functional losses (Table 2). However, further studies using a well-defined sample of patients and with an adequate sample size are required on this issue. Additionally, PUFA supplementation should be studied in different pathologies and at different ages, as well as and the potential adverse effects of long-term DHA supplementation.

Implications for research

DHA should be considered essential for the treatment of certain inborn errors, and the optimal omega 3 LCPUFA dosing requirements for the paediatric age group should be determined. Future researchers should agree on the range of outcome measurements to ensure consistency across trials.

Commentaries

DHA should be considered essential for the treatment of certain chronic diseases such as aminoacidopathies, and other inborn metabolic disorders. In these cases it is important to ensure and adequate intake of foods rich in this fatty acid and to quantitatively determine the DHA requirements for these children to get benefits in health. Since PKU children are generally healthy and have normal energy and fatty acid metabolism, these data lead us to conclude that childhood populations in general require preformed *n*-3 LCPUFA to achieve optimal neurological function.

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Contribution of Gil-Campos M to the manuscript: review of literature and studies in children, and preparation the manuscript.

Contribution of Sanjurjo P to the manuscript: review of studies in children with inborn errors, and revision of the manuscript.

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