2020

Stunting is a recognised problem: evidence for the potential benefits of omega-3 long chain polyunsaturated fatty acids

Setyaningrum Rahmawaty
Universitas Muhammadiyah Surakarta, sr926@uowmail.edu.au

Barbara J. Meyer
University of Wollongong, bmeyer@uow.edu.au

Stunting is a recognised problem: evidence for the potential benefits of omega-3 long chain polyunsaturated fatty acids

Abstract

Stunting remains a major nutritional problem worldwide, especially in middle and low-income countries including Indonesia. A number of nutrition feeding programs to cure stunting is basically addressing the problem of malnutrition. These programs are particularly designed to promote recovery of normal metabolic function and weight gain. The currently available remedial formula for malnutrition contains energy and nutrients to recover their weight, but there is less nutrition that would support optimal functional development of tissues including central nervous system. Research has well documented that sufficient intakes of dietary omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) are important for optimal health throughout the lifespan, especially on brain development and cognition. We review evidence for the role of food sources of these fatty acids on children's health and growth. There is very little work done on remedial formula enriched with n-3 LCPUFA for malnutrition and optimal health, highlighting a need for intervention studies investigating the health benefits of the formula for malnutrition and optimal health, which may prevent stunting but also may promote optimal health.

Keywords

benefits, acids, stunting, recognised, omega-3, long, fatty, chain, polyunsaturated, problem:, evidence, potential

Disciplines

Medicine and Health Sciences

Publication Details

Rahmawaty, S. & Meyer, B. J. (2020). Stunting is a recognised problem: evidence for the potential benefits of omega-3 long chain polyunsaturated fatty acids. Nutrition, 73 110564-1-110564-13.

Title page

Review

Stunting is a recognised problem: evidence for the potential benefits of omega-3 long chain polyunsaturated fatty acids

Running head: Potential benefits of n-3 LCPUFA for stunting

Authors: Setyaningrum Rahmawaty[1], Barbara J Meyer[2,3]

Setyaningrum Rahmawaty (✉)
[1]Department of Nutrition Science, Faculty of Health Science, Universitas Muhammadiyah Surakarta, Indonesia. Jl. A. YaniTromolPos 1 PabelanKartasura, Surakarta, Central Java, Indonesia. Post code: 57102. Tel.: +62 71 717417 Fax: +62 71 715448. Email: setyaningrum_r@ums.ac.id

Barbara J Meyer
[2] School of Medicine, Lipid Research Centre, Molecular Horizons, Faculty of Science, Medicine and Health, University of Wollongong and [3]Illawarra Health and Medical Research Institute, Northfields Ave, Wollongong NSW 2522, Australia. Tel.: +61 (0)2 4221 3459, Fax: +61 (0)2 4221 5945, Email: bmeyer@uow.edu.au.

Role of each author in the work:
SR performed in conceptualization and drafted the manuscript, BJM co-authored the manuscript and were involved in drafted and finalizing the manuscript. All authors contributed to the manuscript, read and approved the final manuscript.

A word count for the entire manuscript (including figures and tables)
9067

Abstract

Stunting remains a major nutritional problem worldwide, especially in middle and low-income countries including Indonesia. A number of nutrition feeding programs to cure stunting is basically addressing the problem of malnutrition. These programs are particularly designed to promote recovery of normal metabolic function and weight gain. The currently available remedial formula for malnutrition contains energy and nutrients to recover their weight, but there is less nutrition that would support optimal functional development of tissues including central nervous system. Research has well documented that sufficient intakes of dietary omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) are important for optimal health throughout the lifespan, especially on brain development and cognition. We review evidence for the role of food sources of these fatty acids on children's health and growth. There is very little work done on remedial formula enriched with n-3 LCPUFA for malnutrition and optimal health, highlighting a need for intervention studies investigating the health benefits of the formula for malnutrition and optimal health, which may prevent stunting but also may promote optimal health.

Keywords: stunting, health benefits, omega-3 long chain polyunsaturated fatty acid

1. Introduction

Stunting defined as height-for-age Z-score <-2.0 according to WHO growth standards remains a major nutritional problem worldwide, especially in middle-income and low-income countries including Indonesia. Stunting occurs in one-third of children under 5 yo and it causes 14% of childhood death in developing countries[1]. According to the Indonesian national basic health research data[2], the national prevalence of stunting in Indonesia was 37.2% which was divided into 18% of stunting (<-2.0 z-score ≥-3.0) and 19.2% of severe stunting (z-score

<-3.0). This number increased from the previous national survey in 2010 and 2007, which were 35.6% and 36.8%, respectively. Following the survey, an annually nutritional status monitoring has been conducted under the coordination of the Ministry of Health of Indonesia and found that in 2015 prevalence of stunting in children under 5 yo was 29%[3], which slightly decreased in 2016 of 27.5%[4], but increased in 2017 of 29.5%[5]. More importantly, the prevalence of stunting is not decreasing.

A number of nutrition feeding programs to treat stunting is basically addressing the problem of malnutrition such as using biofortification, prebiotic foods and remedial formula such as ready to use therapeutic foods (RUTF) that meet WHO standard and formula known as F75 and F100 as well as modification dried skim cotton oil (Modisco). The last two formulas are more familiar in treatment of malnutrition in Indonesia. RUTF are energy dense, micronutrient enhanced pastes with a homogenous mix of lipid rich foods that can be directly given to patient without cooking. The nutritional profile of the RUTF is similar to the WHO-recommended therapeutic milk formula used for inpatient therapeutic feeding programmes. Currently, the availability of RUTF is supported by the UNICEF as a part of community-based management for acute-malnourished children globally[6]. The F-75 is designed to promote recovery of normal metabolic function and nutrition electrolytic balance, hence it is initially admitted to in-patients with no adequate appetite and/or have a major medical complication. In this stage rapid weight gain is precarious, hence patients do not gain weight during this stage. Once the acute condition is stabilized, it is advised to change the diet to F-100 which enables to recover weight and lean body tissue loss[7].

Malnutrition children do not only need diets containing energy and nutrients to recover their weight, but also nutrients that support optimal functional development of tissues including central nervous system. A study by Danaei et al. (2016) concluded that the leading risk worldwide for stunting is fetal growth restriction being defined as term and small of gestational age – 10.8 million cases of stunting out of 44.1 million[8]. Hence sufficient dietary

intake of n-3 LCPUFA are essential to support normal brain development, especially in the critical period including during pregnancy, lactation as well childhood.

The n-3 LCPUFA are involved in various neuronal processes, ranging from regulation of gene transcription to effects on cellular signalling processes[9], and protection against the pathogenesis of retinal diseases[10]. Nutrient deficiency during the brain growth spurt, which is between the last trimester of gestational and the first two years of childhood can damage brain function[11].

In the RUTF as well as F75/100 and Modisco that are being used to date, the n-3 LCPUFA composition is limited (Supplemental Table 1, 2 and 3). In general, intervention studies used the F75/F100 as well as Modisco and RUTF showed an improvement of weight but limited data were reported on height. The efficacy of RUTF has been evaluated in a number of studies for treatment of malnourished children including in Nigeria[12], Malawi[13] and in India[14], and concluded that RUTF significantly improved weight gain of the children. A study in Indonesia using quasy experimental pre-posttest with control group design in outpatient malnourished children aged 6-59 months showed that 5 weeks intervention with F75/F100 increase weight of the children of 507 g compared to control group which only gained 80 g weight[15]. Another study using randomized blinded pre-posttest with control group design of inpatients malnourished children aged 1-3 yo reported that after 6 months intervention, the weight gain of children in the intervention group who received Modisco with elemental milk formula which contains medium chain triglyceride and polymer carbohydrate is significantly higher (1527 g) than in control group given Modisco with regular milk formula (726 g)[16]. Moreover, the data showed that the improvement of weight for height in the intervention group is significantly higher (z-score from -3.59 to -1.57) than in control group (z-score from -3.99 to -2.98)[16].

The effect of long chain PUFA substitution for malnutrition children have been reported in 2 studies by Jones *et al*. (2015)[17] and Hsieh *et al*. (2015)[18]. In study by Jones *et al.* (2015)

reported that malnourished children who had received high-oleic RUTF made with peanut oil from a high-oleic acid peanut cultivar, hence the ratio of linoleic acid (LA) to alpha-linolenic acid (ALA) achieving 1:1 had a significantly better weight-for height (WFH) at the time they recovered from malnutrition compared to children who were fed with standard RUFT that contained 53 times more LA than ALA, although still marginally below average weight-for-height[17]. The levels of n-3 eicosapentaenoic acid (n-3 EPA) and n-6 DPA decreased from 3.2 to 2.4%[1717]. This indicates that the standard RUTF did not support circulating DHA levels. In contrast, the children with high-oleic RUTF showed increased EPA and DPA and protected the decreasing of DHA levels during recovery period. In addition, compared to children receiving RUTF, high-oleic RUTF also induced a lowering of arachidonic acid (AA) levels, indicating that less LA was transformed to AA or that AA was displaced from plasma phospholipids by the increased levels of n-3 LCPUFA[17]. Another study by Hsieh *et al.* (2015) showed that DHA levels in red blood cells membranes significantly decreased after 4 weeks intervention by standard RUTF (from 5.2% to ~4%)[18]. "In the children receiving fish oil, DHA levels in RBC membranes increased over time to a level around 6-8%. Only supplementation with fish oil increased EPA levels, while flax oil-RUTF did not change the overall n-6 to n-3 ratio compared to baseline. This ratio increased with standard RUTF but decreased with the fish oil supplementation to flax oil RUTF[18]. In this paper, we review the potential benefits of n-3 LCPUFA for optimizing growth and development of stunted children.

In this paper we review 1) n-3 LCPUFA metabolism to highlight the importance of n-3 LCPUFA and not rely on the conversion of ALA to n-3 LCPUFA; 2) the health benefits of n-3 LCPUFA (and not ALA) during pregnancy, lactation and throughout offspring growth and development; 3) the recommended intakes and compare those to actual intakes to highlight the importance that we need to increase our intakes of n-3 LCPUFA; 4) how we can

meet these recommended intakes through various n-3 LCPUFA enriched foods and 5) the rationale for the inclusion of n-3 LCPUFA in RUTF which may prevent stunting.

2. n-3 LCPUFA metabolism

Plants are able to synthesise LA (18:2n-6) and ALA (C18:3n-3) but human beings cannot synthesise LA and ALA and they need to obtain these polyunsaturated fatty acids (PUFA) from their diet and therefore they are considered essential fatty acids[19]. The metabolism of these essential PUFA to the long chain PUFA (LCPUFA) including arachidonic acid (AA, 20:4n-6) in the n-6 pathway and eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) from the n-3 pathway, has been well described[20,21] and shown in figure 1. Delta-6 desaturase is the enzyme in the first step of the pathway for both the n-6 and n-3 pathways. Furthermore, delta-6 desaturase is the enzyme that converts tetracosapentaenoic acid (24:5n-3) to tetrahexaenoic acid (24:6n-3), which is then converted to docosahexaenoic acid (DHA, 22:6n-3) via beta-oxidation. Studies have shown that this latter step involving delta-6 desaturase is limited due to the competition of the other fatty acid substrates for the enzyme and hence de novo DHA synthesis is limited[22,23,24,25,26,27]. Therefore DHA may be considered as an essential nutrient[28], or at least a semi-essential nutrient, that is a nutrient that can be synthesised but not in the quantity required for optimal health.

People who consume a vegan or vegetarian diet generally do not consume meat, fish and seafood, so they do not consume preformed n-3 LCPUFA including DHA. Therefore, they need to rely on their ability to metabolise ALA to EPA, DPA and DHA. One study showed that the mean serum levels of EPA and DHA expressed as percent of total fatty acids are much lower in vegans 0.63% and 0.85% respectively compared to non-vegetarians with EPA and DHA levels of 2.33% and 2.25% respectively in Finnish men and women aged 24-

52 years[29]. This is further evidence that consumption of preformed n-3 LCPUFA is better than relying on the conversion of ALA to the n-3 LCPUFA.

Supplementation with 9g LA and 2.8g ALA during pregnancy compared to 10.9g LA as the control, resulted in an approximate 2 fold increase in maternal plasma ALA levels, but there were no increases in maternal EPA, DPA and DHA[30]. This supplementation resulted in a 2 fold increase in neonatal plasma EPA levels, but no increased levels of neonatal plasma DPA and DHA[30]. This study demonstrates that supplementation with the precursor of DHA, namely ALA, does not result in increased DHA in the maternal and the fetal circulation. However, supplementation with preformed DHA (600mg per day from 20 weeks gestation until delivery) resulted in increased maternal and cord blood DHA levels[31], suggesting that consumption of preformed DHA during pregnancy is superior to consuming ALA and then converting ALA to DHA.

In a study of children aged 0-18 years with disorders of amino acid metabolism who consume protein-restricted diets (and hence do not consume pre-formed n-3 LCPUFA e.g. fish or seafood, egg and meat products), their erythrocyte DHA concentrations were 30% lower compared to healthy children, whilst concentrations of arachidonic acid (AA) did not differ between the groups[25].This evidence suggests that these children do not have a problem with the elongation and desaturation enzymes because LA is converted to AA. However, due to high dietary LA levels, the ability to convert ALA to DHA is reduced[25]. In another cross-sectional study of children with allergies, who eliminated food groups such as milk, egg, fish and vegetables, those children had approximately two-fold lower levels of plasma EPA and DHA compared to healthy children, whilst the plasma concentrations of AA and ALA did not differ[32]. These studies suggest that pre-formed n-3 LCPUFA needs to be consumed to fulfil physiological requirements, and that conversion of ALA to n-3 LCPUFA is not adequate for optimal health. Furthermore, evidence from our ancient diet, epidemiology, DHA status

regulation and randomised controlled trials supports that DHA is essential[28], that is, we must consume DHA in our diets.

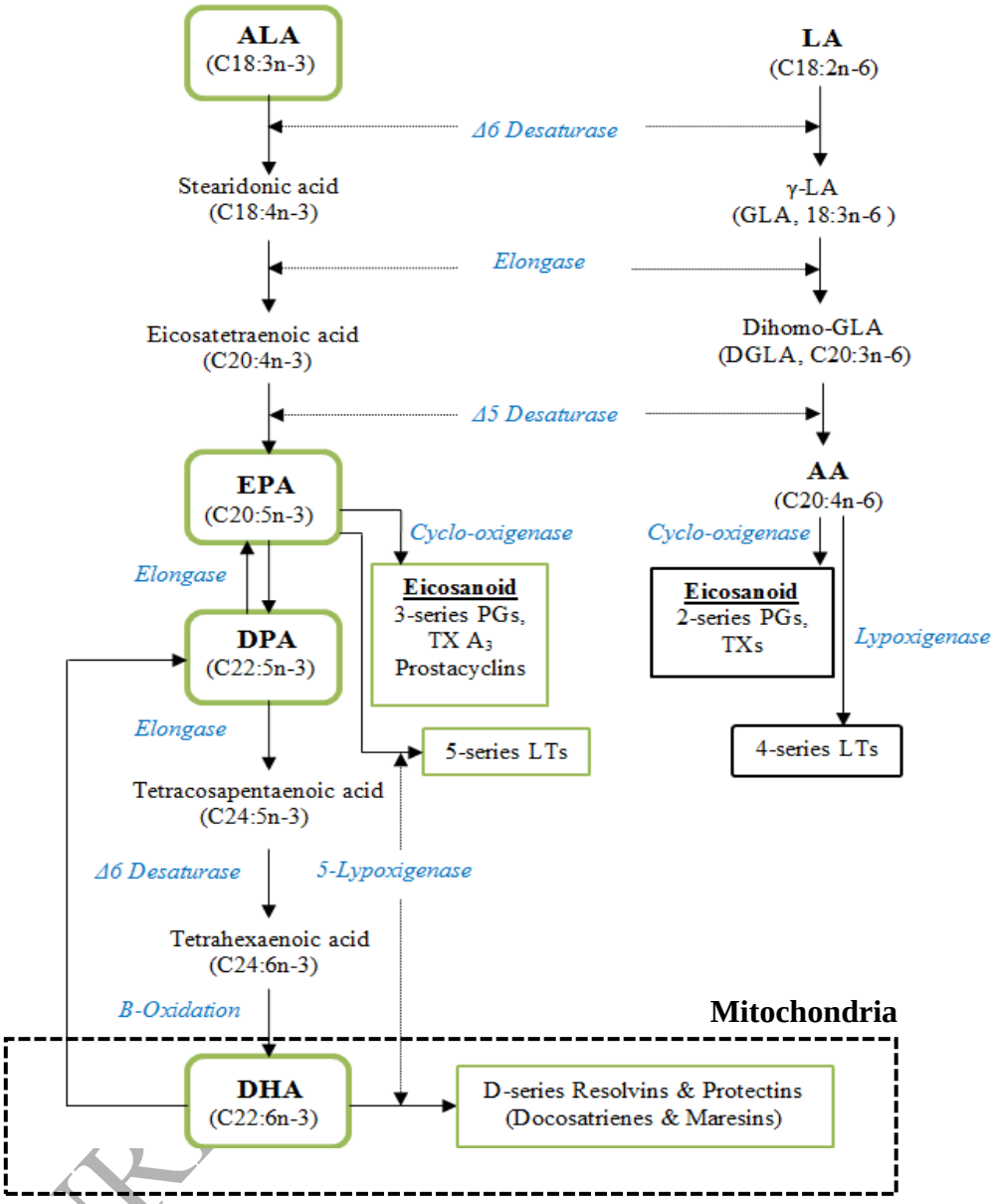

Figure 1: Metabolism of n-3 LCPUFA and eicosanoids, adopted from: Ratnayake & Galli, 2009[20]; SanGiovanni & Chew 2005[21] with some modifications

3. Health benefits of n-3 LCPUFA

3.1 The role of n-3 LCPUFA on neurological development

DHA is a crucial element in the nervous system, which is responsible for development of the sensory, perceptual, cognitive and motor neural system during the brain growth spurt[33Error!

Bookmark not defined.[34]. It is the most fluidizing compound in cell membrane[35], which is fundamental for growing membranes[36].. The need for DHA in very early pregnancy has been shown in a prospective, observational study of human pregnancy where there was a rapid early increase in the maternal rate of change of plasma DHA concentration from 0.1 to 1.6 nmol DHA per millilitre of plasma per day by 29 days post luteinising hormone surge that coincided with the closure of the neural tube[37]. This increased plasma DHA concentration was 2 fold higher in twin pregnancies than in singleton pregnancies, emphasizing the metabolic response to increased demand for DHA at the critical time of the closure of the neural tube[37]. The metabolic response was increased synthesis of DHA from ALA, as delta-6 desaturase correlated with the rate of change of DHA plasma concentration between 18 and 29 days post-LH surge (R^2adj = 0.41, P=0.0002)[37].

The retina, functionally an extension of the brain, is highly enriched in DHA, especially in retinal photoreceptor outer segment disc membranes[38]. DHA is highly accumulated in the brain and retinal photoreceptor cells, forming 10-20 % and >60% of total fatty acid composition in the brain and retina, respectively[39,40]. During the third trimester of gestation (week 26-40), there is a rapid increase (~14.6 mg/week) of accretion of DHA in the fetus[41,42].

3.1.1 DHA from birth to old age

Concentration of DHA in plasma phospholipids and red blood cells rapidly falls by ~50% within 4 months after birth without an exogenous source of DHA, but is maintained by consumption of human breast milk or DHA-fortified formula feeding[43]). The accretion of DHA in the brain continues up to 2 years of age and progressively rises in the cerebral cortex until 18 years of age[44,45] and even life-long[33]. A study has compared brain tissue obtained from individuals ranging from fetal age to 82 years to assess percentages of AA and DHA methyl esters in cerebral cortex ethanolamine glycerophospholipids[46]. Concentrations of AA and DHA in the one month old human infant were roughly equal or 1:1 (16.5% AA and

16.1% DHA) but by the 82nd year the percentage of DHA had more than doubled and increased in proportion to AA, to approximate a ratio of 1:3 (10.3% AA and 33.9% DHA)[46].

3.2 Maternal plasma DHA and LA during pregnancy and offspring growth, weight and adiposity

Increased consumption of n-3 LCPUFA in the maternal diet and increased levels of n-3 LCPUFA in the umbilical cord plasma phospholipids has been associated with lower adiposity in children age 3 years; where the lower adiposity has been shown by lower skinfold thickness, odds of obesity and concentration of leptin, a biomarker of adiposity[47]. Increased fish intake is also associated with lower adiposity but the relationship is weaker than n-3 LCPUFA[47]. The increased n-6 to n-3 ratio in the maternal diet, maternal blood and cord blood is also associated with increased adiposity at 3 years of age, but this is due to reduced n-3 rather than increased n-6[47].

In a cohort longitudinal study DHA was positively associated with height from birth to age 5 years, in that for a 5% level increase, height was 0.63 (0.09, 1.16) cm and 1.29 (0.34, 2.24) cm higher at 1 and 5 years respectively[48]. There were no associations between DHA and weight, BMI and head circumference[48]. In this same study, LA was associated with all measures of fetal growth at 26 and 32 weeks gestation and at birth with exception of birth length and abdominal circumference. LA was not associated with overall post-natal growth as associations faded after one month after birth. LA was increased with fat mass as measured by skinfold thickness and neonatal abdominal adipose tissue volume (measured by MRI) which showed increases in superficial subcutaneous and internal tissues[48]. These results demonstrate that DHA is associated with growth in terms of increased height but not weight (or fatness), whilst plasma LA status during pregnancy is associated with fetal growth, neonatal body size and composition, i.e. fatness; suggesting that DHA is better than LA in terms of growth without increased fatness[48].

3.3 DHA and cognition throughout the lifespan

DHA is important throughout the lifespan, from neurological development and cognition through infancy, childhood, adolescents, adulthood and the elderly which has been reviewed by Stonehouse 2014[49] and Weiser *et al.* 2016[50]. Briefly, people with low habitual intake of n-3 LCPUFA, children who are malnourished and with low literacy ability and older people with mild cognitive decline may benefit the most from consuming DHA[49]. DHA can influence many signalling pathways, enzyme activities, receptor systems, membrane structures and dynamics that results in optimal neurological development, maintenance of the nervous system and slows cognitive decline through aging and thereby having optimal cognition throughout the lifespan[50].

3.3.1 DHA supplementation/consumption during pregnancy and cognitive benefits to infants and children

Studies have shown that supplementation with n-3 LCPUFA during pregnancy and lactation resulted in increased cognition in the offspring. Supplementation with 1183 mg DHA and 803 mg EPA per day during pregnancy and lactation increased children's IQ at 4 years of age[51]. In another study women who consumed more than 340 g of fish per week during their pregnancy, their children had higher verbal IQ at age 8 compared to those children whose mothers consumed no fish during pregnancy[52].

In a longitudinal study design the maternal consumption of DHA during pregnancy was divided into low and high DHA intakes at the median intake point, and this study showed that women with high DHA intakes (approximately 181 mg/d) during pregnancy was associated with increased performance on an object search task in the offspring at 22 months of age, compared to the offspring from women with low DHA intakes (72 mg/d) during pregnancy[53]. This finding is consistent with previous findings. Furthermore, DHA supplementation prevented the reduction in sustained attention at 4, 6 and 9 months of age[31]. Collectively,

these studies have shown that maternal consumption of higher levels of DHA during pregnancy results in increased cognition in the offspring.

3.3.2 DHA supplementation to infants and children and cognitive benefits

Supplementation trials in infants showed improved cognition in the infants. The DIAMOND trial was a 12 month intervention trial commencing in infants aged 1-9 days. These infants were randomised to one of 4 supplemental groups: 0.32% DHA and 0.64% AA; 0.64% DHA and 0.64% AA; 0.96% DHA and 0.64% AA; compared to the control group 0% DHA and 0% AA (i.e. the design was to vary the dose of DHA whilst maintaining AA dose). Combining the 3 DHA dose groups together and comparing to the control group, there was a significant increase in the Mental Development Index (MDI) ($p=0.02$), no significant improvement in cognition ($p=0.08$), and a significant improvement in language ($p=0.01$). There was also a negative correlation between infant red blood cell LA levels and cognition ($r_s=-0.19$ $p=0.039$) and language ($r_s=-0.21$ $p=0.022$) at 4 months of age, and the negative correlation with language persisted until 12 months of age ($r_s=-0.19$ $p=0.041$), suggesting that higher LA levels in detrimental to cognition and language in infants[54]. Data from the DIAMOND trial shows that a balance between DHA and AA is required for optimal neurodevelopmental outcomes[55] with a 1:1 or 1:2 balance of DHA to ARA being optimal and is consistent with the conclusions from a recent review[56]. In the RUTF as well as F75/100 and Modisco that are being used to date, the fatty acids composition is limit, based on our nutritional calculation using the fatty acids coumpound of Indonesian foods as Table 2 and 3. The most fatty acids compound is linoleic acid. Hence it is timely to consider adding DHA and AA into the formula at a ratio of 1:1 or 1:2.

An increase in consumption of food sources containing n-3 LCPUFA e.g. fish, seafood[57,58] and foods enriched with n-3[59,60] has been found to be associated with an improvement of cognitive performance in school children. A randomized placebo-controlled,

double-blind study showed a significant positive association (r^2=0.14, p=0.018) between blood levels of DHA and higher scores on the Peabody Picture Vocabulary Test (PPVT) in healthy preschool children[61]. A six month randomly assigned experimental and control group intervention study has reported that consumption of bread spread with fish-flour suppling 892 mg DHA per week, equivalent to 125 mg or 2 portions of medium fat fish per week significantly increased EPA and DHA, which had a positive impact on verbal learning and memory of African children (age 7-9 y)[62].

Similar findings have been reported in an intervention study of Indonesian and Australian primary school children who received supplementation with multiple micronutrients, as well as EPA plus DHA[63]. Improvement of biochemical markers (e.g. anaemia) has also been reported in a double blind-randomized controlled trial study in primary school Indian children after supplementation with multiple micronutrients and DHA[64]. However, in these two studies, there were no differences seen between the DHA group and placebo. The likely reason for no effect of DHA was that very small doses were used, namely 100mg DHA per day[64] and 88mg DHA per day[64].

More recently, in the Dolab study where higher doses of DHA were used, DHA supplementation (600mg per day for 16 weeks) has shown improvements in the reading ability in school children aged 7-9 years whose initial reading ability was $\leq 20^{th}$ centile and those benefits were more pronounced in those children with initial reading ability was $\leq 10^{th}$ centile[65].

These studies provide evidence that DHA supplementation or the consumption of marine-enriched food not only supported optimal health in school-aged children but also showed improvements in their academic achievement[61,62].

Sufficient intake of n-3 LCPUFA is crucial for supporting normal brain, cognitive and retina development, and protection against neuro-psychiatric disorders. Moreover, the brain keeps producing neurons, even into adulthood[66]. Research in primates and rodents has found

that the brain cortex undergoes highly active synaptic turnover throughout life[67]. This suggests that sufficient intake of n-3 LCPUFA is fundamental, not only during the growth spurt period, but also across the life span.

A number of hypotheses have been proposed to explain the poor learning of individuals with n-3 PUFA deficiency. The n-3 PUFA deficiency results in a significantly decreased number of neurons in the hippocampus, hypothalamus and cortex, brain areas that mediate spatial and serial learning[68], cerebral catecholamines[69], glucose transport capacity and glucose utilization in the brain[70], cyclic adenosine monophosphate (cAMP) level in the hippocampus[71] and capacity of phospholipid synthesis in the brain and hypothalamus[72,73]. The cAMP is a second messenger that is important in many biological processes. Any changes in the levels of catecholamines, glucose, cAMP and phospholipids can pose learning deficits.

3.3.3 DHA supplementation and children and adolescent's behaviour

An Australian cross sectional study in 7-12 year olds with ADHD with and without learning difficulties showed that higher n-6 PUFA predicted poorer word reading, spelling, vocabulary and the lower ability to switch and control attention, whilst n-3 PUFA predicted lower anxiety and shyness[74]. Given the current much higher Australian intakes of n-6 PUFA (6-10 g of LA per day for 2-18 year olds) compared to total n-3 PUFA (1.0-1.6 g per day) which is at least 6 times higher n-6 to n-3 intakes[37], this is of concern. Furthermore, the n-3 LCPUFA intakes were much lower ranging from 0.13-0.21 g per day[37]. However, cross sectional studies do not show cause and effect.

In an intervention trial in the UK, the Dolab study showed that DHA supplementation (600 mg per day for 16 weeks) resulted in improvements in hyperactivity and oppositional behaviour, global scales tapping emotional lability (mood swings) and restless impulsive behaviour as well as total ADHD-type symptoms[65] compared to the control group. Another

intervention trial also showed benefit of 732 mg n-3 LCPUFA supplementation per day and reductions in ADHD symptoms including inattention, hyperactivity and impulsivity[75].

In a review on n-3 deficits and adverse neurodevelopment and childhood behaviours[76], the authors claim that a Western diet contains an imbalance of n-6 and n-3 PUFA which needs to be corrected. Given the low conversion rate of ALA to DHA and hence the essentiality of DHA[28], DHA intakes should be increased for optimal neurological development and optimal health.

3.4 n-3 LCPUFA intakes and recommended intakes

Table 1 showed dietary n-3 LCPUFA intakes of children in many developed and developing countries are reported to be much lower than recommended desirable intakes (Table 2) with an exception is in Japan[77].Intakes of n-3 LCPUFA (EPA plus DHA) in children that did not consume fish was at least twice as high as in those that did consume fish[78]. Fish consumption, even if low, is a major contributor to total n-3 LCPUFA intake. A study in Belgium has reported that fish and seafood were the highest contributors to EPA, DPA and DHA intake (53.5, 42.8 and 48.1%, respectively); however, only 31% of children in this study consumed fish. The mean of fish was 8.6 g/d in total children, and it was approximately 3-fold higher (27.4 g/d) in the seafood consumer[79]. It can be concluded that low fish consumption is the main reason for sub-optimal n-3 LCPUFA intakes of children worldwide.

Table 1. Mean intake of EPA, DPA and DHA of different population studies of children

Country	Reference	Number of children and age	Method	Definition of total n-3 LCPUFA	Fish/ seafood	EPA		DPA		DHA		Total n-3 LCPUFA	
					g/d	mg/d	% of E	mg/d	% of E	mg/d	% of E	mg/d	% of E
Australia	[80]Meyer *et al.* 2003	383 (2-3 y)	24h recalls	Sum of EPA, DPA and DHA	n/a	10	1.6*	5	0.8*	24	3.8*	40	6.3*
		799 (4-7 y)			n/a	19	2.6*	10	1.3*	47	6.3*	76	10*
		739 (8-11 y)			n/a	30	3.3*	17	1.9*	60	6.7*	106	12*
		653 (12-15 y)			n/a	32	3.2*	22	2.2*	63	6.2*	117	12*
		433 (16-18 y)			n/a	41	3.7*	20	1.8*	77	6.9*	138	12*
US	[81]Ervin *et al*., 2004	962 (6-11 y) 2208 (12-19 y)	1d x 24h recall		n/a n/a	10 (0) 20 (0)	n/a n/a	10 (0) 10 (0)	n/a n/a	40 (10) 50 (10)	n/a n/a	n/a n/a	n/a n/a
Australia	[82]Howe *et al.* 2006	1921 (2-11 y)	24h recalls	Sum of EPA, DPA and DHA	n/a	32	n/a	32	n/a	46	n/a	110	n/a
		1086 (12-18 y)			n/a	57	n/a	63	n/a	75	n/a	195	n/a
Chinese	[83]Barbarich*et al*. 2006	196 (1-5 y)	3 x 24h recall		n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	30± 40 § n/a	n/a n/a	n/a n/a	n/a n/a
Belgium	[79]Sioen *et al.* 2007	661 (2.5-6.5 y)	Parentally reported 3d EDR	Sum of LNA, EPA, DPA and DHA	8.6‡ n/a	25 (1-21)§ §	n/a n/a	10 (2-10)§ §	n/a n/a	47 (5-46)§ §	n/a n/a	941 (730-1100)§ §	n/a n/a
German	[78]Sichert-Hellert *et al.* 2009	1024 (2-18 y)	3d weighed records (yearly)	Sum of EPA and DHA	5.4-13.9††	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	n/a n/a	42-141 n/a	0.04-0.05%
Guatemalan	[84]Bermudez *et al*., 2010	219 (8-12 y, LSES) 230 (8-12 y, HSES)	Single pictorial 24h record	N/A	n/a n/a	8 (2)§† 10 (2)§†	n/a n/a	n/a n/a	n/a n/a	n/a n/a	32 (2) §† 32 (2) §†	n/a n/a	n/a n/a

EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, omega-3 long chain polyunsaturated fatty acid; LNA, linolenic acid; LSES, Low social economy status; HSES, high social economy status; n/a, not available; *, mg/d/energy (MJ); §,Mean ± standard deviation; §§, Mean (inter quartile range); §†, Mean ± SEM; ∞, Mean ± median;††, The lower and the higher mean of all age groups; ‡ Seafood

3.4.1 Infant and children n-3 LCPUFA intakes

DHA intake in children aged 24-35 months (n=221) and 36-48 months (n=236) from rural Bangladesh was 30 mg and 20 mg respectively[85]. Total marine intakes (i.e. EPA and DHA intakes) were 150 mg per day in 404 children aged 1-11 years from Denver Colorado in the USA[86]. The total mean n-3 LCPUFA (EPA, DPA and DHA) intake in Australia was approximately 150 mg per day in 2-12 years olds[37]. These infants and children's intakes are below those recommended intakes as shown in Table 2.

The n-3 LCPUFA are involved in various neuronal processes, ranging from regulation of gene transcription to effects on cellular signalling processes[9], and protection against the pathogenesis of retinal diseases[33]. Nutrient deficiency during the brain growth spurt, which is between the last trimester of gestational and the first two years of childhood can damage brain function[71].

Table 2. Recommended Intakes for babies, infants, children and adolescents per day (unless otherwise stated) for EPA and/or DHA

	0-6 mths	6 mths-3 yrs	>3-9 yrs	>9-18 yrs
Europe				
European Food Safety Agency (authoritative body)[87]		100mg DHA[#]	250mg EPA and DHA[^]	250mg EPA and DHA
France				
The French Food Safety Agency (AFSSA) (authoritative body)[88]	0.32% of fats from DHA EPA<DHA	70mg DHA	125mg DHA 250mg EPA and DHA	250mg DHA 250mg EPA and DHA
Netherlands				
Health Council of the Netherlands (authoritative body)[89]	20mg DHA/kg/day* (70mg DHA if 3.5kg 145mg DHA if	n-3 fatty acids from fish 15-20mg/kg/day 115-154mg (6mth)	n-3 fatty acids from fish 15-20mg/kg/day 228-490mg (G) 262-560mg (B)	n-3 fatty acids from fish 15-20mg/kg/day 490-980mg (G) 560-1260mg (B)

	7.2kg)	210-280mg (3yrs)		
Scandinavia Nordic Council of Ministers (authoritative body)[90]		n-3 fatty acids at least 1% of energy[δ] n-3 fatty acids at least 0.5% of energy[γ]	n-3 fatty acids at least 1% of energy	n-3 fatty acids at least 1% of energy
Range DHA	70-145mg DHA	70-100mg DHA	125mg DHA	250mg DHA
Range EPA and DHA		115-280mg EPA and DHA	250-560mg EPA and DHA	250-1260mg EPA and DHA

[#] For age range 7mths to 2 years; [^]For age range 2 yrs to 9 yrs; * For age range 0-5mths; [δ]For age range 6-11 mths; [γ]For age range 12-23 mths; G is girls; B is boys

3.4.2 Pregnant women DHA intakes

Table 3 shows recommended desirable intakes of dietary n-3 LCPUFA for pregnant and lactating women. The vast majority of Australian women of child bearing age are not meeting the recommended DHA intake of 200 mg per day and only the 90th centile of DHA intakes meets the recommended intake of 200 mg DHA per day[91]. Even Australian pregnant women are only averaging 99 mg DHA per day and the median DHA intake is 75 mg per day[92] which is less than half the recommended DHA intake. Other countries also do not meet the recommended 200 mg DHA intakes per day, such as the USA 110 mg DHA mid pregnancy and 80 mg DHA one month prior to delivery[93], USA 77 mg DHA per day[94], China 10 mg DHA per day[95], UK 80-90 mg DHA per day[53]. Countries that have a high intake of fish/seafood do meet the recommended intake of 200 mg DHA per day such as Japan 227-238 mg DHA per day[96] and Korea 300 mg DHA per day[97], highlighting the importance of regular fish/seafood consumption to meet the recommended intake of 200 mg DHA per day.

To meet the recommended 200 mg DHA per day intakes a pamphlet and shopping card was developed for pregnant women and showed that it was useful, desired by pregnant women and also resulted in increased DHA intake[98]. The pregnant women found that the pamphlet expanded their knowledge about the benefits of DHA, food sources of DHA and practical dietary suggestions to meet the recommended 200 mg DHA per day[98].

Table 3. Recommended DHA Intakes per day for pregnant and lactating women

	Pregnant and Lactating women
Global Organisations	
International Society for the Study of Fatty Acids and Lipids (ISSFAL)[99] (expert scientific organisation)	>200mg DHA
World Association of Perinatal Medicine[100] (expert scientific organisation)	200mg DHA
Countries:	
Austria	
Austrian Society for Nutrition[101] (expert scientific organisation)	>200mg DHA
Belgium	
Belgian Superior Health Council (authoritative body)[102]	250mg DHA
Europe	
European Food Safety Agency (authoritative body)[87]	100-200mg DHA in addition to normal adult recommendation (= 250mg EPA and DHA)
The PeriLip and EARNEST projects of the European Commission[103] (expert scientific organisation)	200mg DHA
France	
The French Food Safety Agency (AFSSA) (authoritative body)[88]	250mg DHA 500mg EPA and DHA
Germany	
German Society for Nutrition[101] (expert scientific organisation)	>200mg DHA
Switzerland	

Swiss Society for Nutrition Research / Swiss Nutrition Association[101] (expert scientific organisation)	>200mg DHA
United States of America	
March of Dimes[104] (expert scientific organisation)	200mg DHA
Overall	≥200mg DHA

3.4.3 Global n-3 LCPUFA intakes

Micha and colleagues reported the global, regional and national consumption levels of various fats including the seafood n-3 LCPUFA from 266 country specific nutrition surveys[105]. The n-3 LCPUFA intakes ranged from 5 to 3,886 mg per day with an overall average of 163 mg per day. Only 18.9% of the estimated world's population met the recommended intake of 250 mg per day of EPA and DHA[105]. Given the evidence above, we need to consider adding n-3 LCPUFA in the diet or formula to promote optimal growth and development of malnutrition including stunting children.

3.5 Breastmilk composition

The quality of fatty acids in breastmilk has changed considerably over the past 30-40 years[106]; where LA has doubled (from 7% to 14% of total fatty acids) and DHA has halved (from 0.4% to 0.2% of total fatty acids). This dramatic change in breastmilk fatty acid composition is likely to be reflective of our dietary intakes of these fatty acids over this period of time. Given the benefits of DHA on mental development index and language[52], this decrease in DHA in breastmilk is of great concern. Furthermore, given that high LA levels in infants correlates negatively with language and cognition, this increased LA in breastmilk is also of great concern.

3.6 The role of n-3 LCPUFA in chronic-diseases prevention

The progression of risk factors for chronic diseases such as cardiovascular diseases (CVD) that are associated with age can be either delayed or accelerated as a function of lifestyle choice[107]. Dietary habits in childhood influence the development of an individual's CVD risk profile in later life[108], whilst, early intake of n-3 LCPUFA has shown beneficial effects regarding the prevention of CVD in later life[57,58]. Several randomized double-blind studies in children aged between 2-18 years have demonstrated beneficial outcomes for a lower risk of development of chronic diseases[57, 106,107,108,109,110]and a better CVD risk factor profiles in children following n-3 LCPUFA supplementation[111-113]. Low concentrations of DHA have been found to be associated with increased concentrations of C-reactive protein (CRP), a sensitive predictor of CVD risk[114], in overweight teenagers[115]. Conversely, an increased intake of n-3 LCPUFA has been shown to modulate vascular functions and inflammatory markers, including lymphocytes, monocytes and level of TNF-α, interleukin (IL)-1β and IL-6, in obese adolescents[107]. A low n-3 index, the sum of erythrocyte EPA and DHA, expressed as a percentage (weight/weight) of total fatty acid concentrations in red blood cell membranes (but not in plasma)[116],has also been found in obese school age children and is associated with insulin resistance[117]. The n-3 index is considered as a potential biomarker that can identify persons at risk for coronary heart disease in adults[118,119]. A value of less than 4% is categorized as high risk while >8% is categorized as low risk[120118]. It has been reported that higher intake of EPA and DHA through fish consumption is associated with higher n-3 index in healthy adolescents and reduced CVD factors in boys[121].

Decrease in DHA levels was associated with increased stearoyl-CoA desaturase (SCD), a desaturating enzyme that modulates fatty acid composition and might

contribute to development of metabolic syndrome in obese children[122]. A reduction in biomarkers of CVD risk namely E-selectin and inter-cellular adhesion molecules (ICAM)-1 has also been observed in healthy children aged 8-14 years following n-3 LCPUFA supplementation[123]. E-selectin and ICAM-1 are members of cell adhesion molecules that are involved in the inflammatory development to endothelial damage, such as seen in CVD[124,125]. Additionally, improvement in endothelium-dependent flow-mediated dilation (FMD) of the brachial artery after DHA supplementation (1.2 g/d for 6 weeks) has also been reported in hyperlipidemic children at risk for early heart disease[126]. Supplementation of 900 mg/d n-3 LCPUFA for a month has been reported to have synergistically reduced insulin resistance together with weight loss in obese at-risk children. The mechanistic effect may be through an increased production of adiponectin and a decrease in inflammatory biomarkers such as TNF- α[127].

An intervention study in healthy children (8-14 y) has reported that consumption of 600 mL/d milk enriched with fish oil, oleic acid, minerals and vitamins (Puleva Max containing fish oil, EPA=10 mg/100 mL and DHA=20 mg/100 mL, n-3=35 mg/100 mL; oleic acid; carbohydrates (sugar and honey); vitamins: A as retinyl ester, B1, B2, B3, pantothenic acid, B6, biotin, folic acid, B12, C, D, and E; minerals: calcium, phosphorus, zinc) that was low in saturated fatty acids reduced incidence of endothelial cell activation, a CVD risk biomarker[128].

Therefore it can be suggested that sufficient intake of n-3 LCPUFA during childhood and adolescence is not only necessary for supporting normal cognitive development during childhood but also appears to reduce risk factor for cardiovascular disease, a disease that progresses throughout life.

4. Foods enriched with n-3 LCPUFA

In countries with traditionally low fish consumption as well as for certain individuals or communities who may not be suitable or convenient to eat fish, n-3 enriched foods could play an important role in meeting the recommendations for n-3 LCPUFA. Ovolacto vegetarian may obtain a limited amount of n-3 LCPUFA from eggs, milk and dairy products, while vegan must rely on *in vivo* biosynthesis of these fatty acids from ALA which it depend on the ratio of ALA and LA from the diet[129]. An international recommendation of consumer's need of a daily 500 mg of n-3 LCPUFA for each person have been calculated and showed that current global fish harvest will not supply this recommendation[130]. Therefore alternative sources are required to meet the recommendation for n-3 LCPUFA for optimal health.

One method to increase n-3 LCPUFA is to incorporate the main sources of n-3 LCPUFA into regularly consumed foods. These include enrichment foods with fish oils, single cell micro algal oils and/or biomass, and under-utilized marine sources e.g. krill[131]. A number of n-3 enriched foods with the alternative source of n-3 and fish oil e.g. breads, eggs, milk and yoghurt can be found in marketplace in Australia and in many countries such as Europe, Canada and South America[25].

It has been reported that regular consumption of a variety of n-3 fortified foods (8 meals/day) providing 50 and 150 mg EPA plus DHA per serving increases the daily n-3 LCPUFA intake of Australian adults from 100-200 mg/d to 1000 mg/d[132], double the recommended n-3 LCPUFA intakes. Furthermore, an increase in n-3 LCPUFA concentration in erythrocytes by 35 and 53% at 3 and 6 months respectively, after supplementation, which is equivalent to an n-3 index of increased from 4 to 7% over 6 months study, placing these subjects in lower risk for cardiac death[133].These incensements have also been reported to be associated with CVD risks reduction and

positively associated with arterial compliance assessment and negatively associated with serum CRP and urinary 11-dehydro-TXB2 excretion[133].

Food matrix or carrier food can influence bioavailability and possible physiological effects of n-3 LCPUFA in the systemic circulation or the target organ[134]. A mini review of currently available published studies on the influence of consumption of products enriched with n-3 LCPUFA[135] demonstrated a comparable bioavailability between foods enriched with n-3 and fish oil supplements. The bioavailability and health effects of food products that are potentially enriched with n-3 LCPUFA are presented in the following part.

4.1 Bread enriched with n-3

Bread is a staple for large numbers of people, especially in Western countries such as in Australia. Enrichment of bread with n-3 LCPUFA may have a critically important role because the typical Western diet, which is relatively high in n-6 and low in n-3[8]. Bread is an ideal medium for n-3 because the CO_2 produced during dough fermentation protects the oil from oxidation, especially while it is exposed to high temperatures during baking[136]. Furthermore, baking did not give rise to oxidation of polymerization of fatty acids[137].

Liu *et al*. (2001)[138] reported an increase in plasma levels of EPA, DHA and total n-3 FA has been reported of hyperlipidaemia subjects after 2 and 4 weeks by consuming 93 g bread per day containing 1.3 g stable fish oil. Furthermore, the authors also reported a decrease in triglycerides and malondialdehyde (MDA), one of several potential marker of oxidative stress or lipid peroxidation[139,140], and an increase in HDL-cholesterol in plasma[138].

Microencapsulated fish oil incorporated into bread and also biscuits and soup (providing 0.9 g n-3 PUFA intake per day) was equally effective to that from fish oil capsules (three 1-g capsules per day) in increasing platelet EPA and DHA of healthy females, after 4 weeks intervention[137]. Consumption of a low dose of n-3 LCPUFA supplemented in bread (20 mg of n-3 LCPUFA per slice, derived from microencapsulated tuna oil on average 6 to 8 slices per day for 3 weeks significantly increased concentration of total n-3LCPUFA (EPA, DPA, DHA) from 2.0 to 2.4% in total plasma and from 3.9 to 4.3% in PL fraction[141].

Microencapsulation of fish oil into food products such as bread has advantages including: 1) microencapsulation can convert fish oils to powder form, thereby increasing the number and type of food into which it can be incorporated; 2) microencapsulation, in minimising oxidation mainly by avoiding contact between the fish oil and oxygen in the air, has the potential to extend the shelf-life of fish oil and also to improve the palatability of fish oil-enriched food products. The palatability of bread was reported to be good and appearing to be the best vehicle for increasing daily EPA and DHA intakes[141.]

4.2 Eggs enriched with n-3 LCPUFA

Eggs can be enriched with n-3 LCPUFA by the addition of fish meal or seed oils (flax or rapeseed) to animal feeds, and this also increasing the n-3 PUFA in chicken meat[142,143]. Chicken are able to convert ALA to DHA better than human[144].

The food enriched with n-3 consumption demonstrates some positively affects in the plasma lipid profile in humans. Consumption of two eggs enriched with n-3 PUFA for 18 days in healthy volunteers significantly increased in HDL-C and decreased in plasma triglyceride, and tends to elevate ratio of HDL-C/TC and HDL-C/LDL-C[145]. A

study in normolipidemic volunteers eating four n-3 PUFA eggs (from flaxseed-fed hens) per day for 2 weeks showed that no significant changes were observed in concentration of total cholesterol, HDL-cholesterol, or plasma triglyceride, and a significant increase in DHA (33%) and total n-3 PUFA and significant decrease in ratio n-6 to n-3 FA in the blood platelet phospholipids[146]. Two weeks consumption of one egg enriched with n-3 per day (from hens fed containing 10% flaxseed) did not significantly increase concentration of plasma total LDL and LDL cholesterol and triglyceride compared with regular egg, and there was a trend of slightly lower concentration of LDL cholesterol[147]. A study in hypercholesterolemic subjects for 6 weeks showed that consumption of 2 eggs enriched with n-3 per day has no effect in total LDL-C[148].

A randomized double-blind, cross-over study of hypercholesterolemic patients has reported that eating two eggs enriched with n-3 from hens fed flaxseed providing 217 mg of DHA and 629 mg total n-3 LCPUFA per day, significantly increased in EPA plus DHA levels in serum phospholipid by 23%, and significance correlated to a reduction of fatal ischemic heart diseases risk and did not change levels of lipid and total serum cholesterol[149]. A double-blind, cross-over study in healthy individual with an age over 45 years concluded that consumption of one egg enriched with n-3 (from hens fed rapeseed oil, and the amount of ALA, EPA and DHA are 9.3, 0.2 and 1.5% in one egg respectively) increased ApoA1 and decreased ApoB/ApoA1 ratio, and had no negative impact on blood lipids and inflammatory markers[150]. Apo-A1 is a major component of HDL that is responsible for carrying cholesterol from arteries, whilst Apo-B is responsible for carrying cholesterol to tissues, and they are accepted as superior markers of CVD risk[151]. It has also been reported that a high Apo-B/Apo-A1 ratio was associated with metabolic syndrome in obese children[152].

An increase in serum lutein, an antioxidant that protects the macula from light-initiated oxidative damage[153] has been found in healthy adults[154] and in healthy lacto-ovo-vegetarian (LOV) adults[155] following intervention by consumption of n-3 enriched eggs. Lutein is highly concentrated in the human eye, in the macula, retina fluid, rod outer segment membranes and the lens of the eye. Together with DHA, lutein can be used for preventing the risk of age-related macular degeneration[156].

4.3 Milk enriched with n-3 LCPUFA

Milk is a very efficient carrier for fat absorption, due to high dispersion of fat milk in micelles facilitating in a large surface for absorption[157]. Bioavailability of microencapsulated fish oil incorporated into milkshake[158] has been shown similar to that from fish oil capsules after 4-week intervention. Semi-skimmed milk containing n-3 fatty acids from fish oil supplying 0.3 g of DHA plus EPA per day increase in plasma concentrations of DHA plus EPA (30% on average) together with a significant 19% reduction in fasting plasma triacylglycerols levels after 6 weeks intervention[157]. Consumption of 500 mL of the n-3 enriched milk providing DHA and EPA 0.13 g and 0.2 g respectively significantly increased plasma levels of DHA and EPA (on average 30%) and decreased in plasma levels of homocysteine (13%), after 2 months intervention[159]. In another study, an average inclusion of 300 mg EPA plus DHA in milks produced a 25-50% improvement in plasma levels of fatty acids after a minimum period of 6 weeks[113].

Blood lipid effects of the n-3 enriched milks have been reported in healthy children and adults, and also in CVD patients[143]. All the studies concluded that consumption of milk substituted with EPA plus DHA, PUFA and oleic acid for more than 6 weeks resulted in sustainable reductions blood lipid concentration including total

cholesterol (range 4-11%) and LDL-cholesterol (6-20%), particularly when baseline values before intervention were increased[143]. Another study by Romeo *et al*.[123] reported that consumption of milk enriched with fish oil EPA plus DHA for 5 months significantly decreased endothelial cell activation indices namely ICAM-1 in healthy children aged 8-14 y. Soluble ICAM-1 is associated with future risk factors for atherosclerosis in children[160], while high concentration of serum E-selectin is correlated with the development of atherosclerosis and increase in CVD risk in adults[161]. This suggests that consumption of food enriched with n-3 may reduce CVD risks.

Other study reported that cconsumption of 200 mL milk fortified with 2 g fish oil twice a week for six months (provided 200 mg EPA plus 1 g DHA each day) significantly increased n-3 LCPUFA in plasma phosphatidylcholine and decreased episode and duration of illness, particularly upper respiratory tract infection such as rhinitis, cold and influenza in Thai school children (9-12 y)[162].

4.4 Yoghurt enriched with n-3 LCPUFA

Yoghurt enriched with n-3 LCPUFA is possibly a better way to deliver compared to milk. Compared to milk enriched with fish-oil, yoghurt enriched with fish-oil demonstrated less susceptibility to oxidation[163], thereby reducing fishy odours and flavour[164]. Antioxidant peptides released during the fermentation of milk by lactic acid bacteria and/or the lower oxygen content of yoghurt might responsible to the higher oxidative stability of yoghurt[165]. Technique in adding fish oil for example net fish oil emulsion seemed to increase the stability of yoghurt than those with fish-oil-in-water emulsion[163].

A bioavailability study in humans demonstrated that yoghurt drink was the best matrix compared to fitness bar, butter and bread containing fish oil for providing fast absorption of EPA and DHA[166] which might be due to the preformed emulsions.

4.5 Other foods enriched with n-3 LCPUFA

A number of food products such as dip and juice have also been enriched with n-3 LCPUA and showed positive effect to lipid profile. Consumption n-3 PUFA-enriched dip 100 g/d supplying 1.3-1.4 g of n-3 PUFA(sum of ALA, EPA, DPA and DHA) for 6 weeks increased EPA, DPA and DHA concentrations in plasma lipids by 117, 15 and 80% respectively, significantly reduced plasma triglyceride level (1.93 mmol/L to 1.27 mmol/L) and increased HDL-cholesterol in people with type 2 diabetes mellitus[167]. A study in healthy children age 4-12 y in US reported an increase in phospholipids DHA contents by 40-50 and 65-70% after 6 weeks supplementation of 180 mL juice/d fortified with 50 and 100 mg/d microencapsulated-algal DHA respectively[62]. Algal oil provide an equivalent amount of DHA to bloodstream as cooked salmon, whereas bioavailability of 600 mg/d DHA from salmon or algal oil are equivalent for incorporation into plasma erythrocytes and phospholipids[168].

5. Inclusion of n-3 LCPUFA in RUTF rationale

In this review, we have reviewed the evidence that humans are able to synthesise the n-3 LCPUFA from ALA, albeit limited, especially to DHA. Therefore, rather than relying on the conversion of ALA to n-3 LCPUFA including DHA, it is better to consume preformed n-3 LCPUFA. We have provided evidence that n-3 LCPUFA are vital 1) for neurological development, especially DHA; 2) from birth to old age, especially DHA; 3) for cognitive development, especially DHA; and 4) to prevent chronic disease in

children. However, our current n-3 LCPUFA intakes globally are not meeting the global recommended intakes. We are consuming too much LA and not enough n-3 LCPUFA and this is reflective in current breastmilk composition. The majority of this evidence reported in this review has been conducted in children that are not considered stunted, as there is a lack of research in this specific population. There are two studies that have shown beneficial effects of LCPUFA substitution for children with malnutrition[17,18] but more research is warranted in those suffering stunting. Given that 1) DHA consumption during pregnancy results in increase cognition in the offspring; 2) DHA is better than LA in terms of growth (i.e. increased height but not weight or fatness), whereas LA increases adiposity but not height; 3) higher LA intake in the first 12 months of life results in reduced cognition and reduced language in infants and 4) DHA supplementation in children results in increased cognition: it is timely that we consider adding n-3 LCPUFA, especially DHA to RUTF. Furthermore, to ensure optimal benefit we recommend replacing LA in RUTF with DHA and AA in a ratio of 1:1 or 1:2 (DHA:AA ratio). More research is warranted on RUTF containing DHA and AA with little or no LA in preventing stunting.

6. Conclusions

The n-3 LCPUFA must be obtained from pre-formed n-3 LCPUFA (not from the conversion from ALA to DHA) which are provided in fish. However, numerous barriers to consume fish lead to a failure to meet the reference values for fish consumption. A low dose of enrichment food with n-3 LCPUFA can be used as a long-term strategy to achieve the health benefits of n-3 LCPUFA for optimal health, including for stunted children. Due to the remedial formula to treat stunted children currently lacking on n-3 LCPUFA with the primary fatty acids compound being LA, it

is timely to consider adding DHA and AA into the formula at a ratio of 1:1 or 1:2. Food enrichment with specific nutrients such as n-3 enriched food can lead to relatively rapid changes in the specific nutritional status of a community, and is a cost-effective public health intervention. However the items need to be consumed in adequate amount by a large proportion of target individuals in the population and the levels of fortification must be high enough to substantially increase the intakes.

Declaration of Competing Interest
The authors have no conflict of interest to declare.

Acknowledgement

We are grateful to the Ministry of Research, Technology and Higher Education of the Republic of Indonesia for World Class Professor Program, contract number 123.40/D2.3/KP/2018.

7. References

1 Danaei G, Andrews KG, Sudfeld CR, FinkG, McCoy DC, Peet E, *et al*. Risk Factors forChildhood Stunting in 137 Developing Countries: AComparative Risk Assessment Analysis at Global,Regional, and Country Levels. PLoS Med 2016; 13(11):e1002164. doi:10.1371/journal.pmed.1002164.

2 Riskesdas. *Riset Kesehatan Dasar 2013* (Basic Health Research). Jakarta: Badan Litbangkes, Depkes RI 2013.

3 Ministry of Health. *Buku Saku Pemantauan Status Gizi dan Indikator Kinerja Gizi Tahun 2015*(Pocket Book on Nutrition Status Monitoring and Nutrition Performance Indicators in 2015). Jakarta: Direktorat Jenderal Kesehatan Masyarakat Kementerian Kesehatan RI 2016.

4 Ministry of Health. *Hasil Pemantauan Status Gizi (PSG)dan Penjelasannya Tahun 2016* (Results of Nutritional StatusMonitoring and its Explanation in 2016). Jakarta: Direktorat Gizi Masyarakat Direktorat Jenderal Kesehatan Masyarakat Kementerian Kesehatan 2017.

[5] Ministry of Health. *Hasil Pemantauan Status Gizi (PSG) Tahun 2017*(Results of Monitoring Nutritional Status in 2017). Jakarta: Direktorat Gizi Masyarakat, Direktorat Jenderal Kesehatan Masyarakat, Kementerian Kesehatan 2018.

[6] Unicef. Position paper Ready-to-use Therapeutic Food for cildren with severe acute malnutrition. Unicef 2013.

[7] WHO. Management of a severe malnutrition: a manual for physicians and other senior health workers. 1999. Available at: http://apps.who.int/iris/bitstream/handle/10665/41999/a57361.pdf;sequence=1

[8] Danaei G, Andrews KG, Sudfeld CR, Fink G, McCoy DC, Peet E, *et al.* Risk Factors for Childhood Stunting in 137 Developing Countries: A Comparative Risk Assessment Analysis at Global, Regional, and Country Levels. PLoS Med 2016; 13(11): e1002164. doi:10.1371/journal.pmed.1002164.

[9] Schuchard JP, Huss M, Stauss-Grabo M, Hahn A. Significance of long-chain polyunsaturated fatty acids for the development and behaviour of children. Eur J Pediatr 2010;169:149-164.

[10] Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 1968;9:570-579.

[11] Nanjo A, Kanazawa A, Sato K, Banno F, Fujimoto K. Depletion of dietary n-3 fatty acid affects the level of cyclic AMP in rat hippocampus. J Nutr Sci Vitaminol 1999;45:633-641.

[12] S Isanaka, N Nombela, A Djibo, M Poupard, D Van Beckhoven, V Gaboulaud *et al.* Effect of preventive supplementation with ready-to-use therapeutic food on the nutritional status, mortality,and morbidity of children aged 6 to 60 months in niger: a cluster randomized trial. Jama 2009;301(3):277-285.

[13] MA Ciliberto, H Sandige, MJ Ndekha, P Ashorn, A Briend, HM Ciliberto *et al.* Comparisonof home-based therapy with ready-to-use therapeutic food with standard therapy in the treatment of malnourished Malawian children: a controlled, clinical effectiveness trial. Am J Clin Nutr 2005;81(4):864-870.

[14] VR Wasnik, M Rathi. Effect of locally made ready-to-use therapeutic food (Mushpro Health Drink Powder–MHDP) for treatment of malnutrition on children aged 6 to 72 months in tribal area of Amravati District of Maharashtra, India: A randomized control trial. Int J Collaborative Research on Internal Medicine & Public Health (IJCRIMPH) 2012;4(7).

[15] Sulistiyawati, Rustina Y, Hastono SP. Pemberian diet formula 75 dan 100 meningkatkan berat badan balita gizi buruk rawat jalan (Giving diet F75 and 100 improved weight of malnourished outpatien children under 5 yo). Jurnal Keperawatan Indonesia 2012;15:159-164.

[16] Irawan R. Perbedaan kecepatan kesembuhan anak gizi buruk yang diberi modisco susu formula dan modisco susu formula elemental di RSU Dr Soetomo (Difference of recovery speed of malnourished children given modisco milk formula and modisco milk-elemental at Dr Soetomo hospital). Thesis. Program Studi Ilmu Kesehatan Masyarakat. Program Pasca Sarjana Universitas Airlangga 2005.

[17] Jones KD, Ali R, Khasira MA, Odera D, West AL, Koster G *et al*. Ready-to-use therapeutic food with elevated n-3 polyunsaturated fatty acid content, with or without fish oil, to treat severe acute malnutrition: a randomized controlled trial. BMC Med 2015;13:93.

[18] Hsieh JC, Liu L, Zeilani M, Ickes S, Trehan I, Maleta K *et al*. High-oleic ready-to-use therapeutic food maintains docosahexaenoic acid status in severe malnutrition. J Pediatr Gastroenterol Nutr 2015;61(1):138-143.

[19] Das UN. Essential fatty acids: biochemistry, physiology and pathology. Biotec J 1 2006:420-439.

[20] Ratnayake WMN, Galli C. Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism: A background review paper. Ann Nutr Metab 2009;55:8-43.

[21] SanGiovanni JP, Chew EY. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research 2005;24:87-138.

[22] Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277-288.

[23] Pawlosky RJ, Hibbeln, Novotny JA, Salem N. Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. J Lipid Res 2001;42:1257-1265.

[24] Burdge GC, Finnegan YE, Minihane AM, Williams CM, Wootton SA. Effect of altered dietary n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13 C]α-linolenic acid to longer-chain fatty acids and partitioning towards β-oxidation in older men. Br J Nutr 2003;90:311-321.

[25] Vlaardingerbroek H, Hornstra G, de Koning TJ, Smeitink JAM, Bakker HD, de KlerkHBC *et al*. Essential polyunsaturated fatty acids in plasma and erythrocytes of children with inborn errors of amino acid metabolism. Mol Genet Metab 2006;88:159-165.

[26] Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ, Holub BJ. Effect of dietary linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. Lipids 1993;28: 811-817.

[27] Fokkema MR, Brouwer DA, Hasperhoven MB, Martini IA, Muskiet FA. Short-term supplementation of low-dose gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), or GLA plus ALA does not augment LCP omega 3 status of Dutch vegans to an appreciable extent. Prostaglandins Leukot Essent Fatty Acids 2000;63:287-292.

[28] Muskiet FAJ, Fokkema MR, Schaafsma A, Boersma ER, Crawford MA. Is docosahexaenoic acid (DHA) essential? lessons from DHA status regulation, our ancient diet, epidemiology and randomized controlled trials. J Nutr 2004;134:183-186.

[29] Elorinne A-L, Alfthan G, Erlund I, Kivimäki H, Paju A, Salminen I, *et al*. Food and Nutrient Intake and Nutritional Status of Finnish Vegans and Non-Vegetarians. PLoS ONE 2016;11(2):e0148235. doi:10.1371/journal.pone.0148235.

[30] de Groot RHM, Hornstra G, Houwelingen AC, Roumen F. Effect of α-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. Am J Clin Nutr 2004;79:251-260.

[31] Carlson SE, Colombo J, Gajewski BJ, Gustafson KM, Mundy D, Yeast J *et al*. DHA supplementation and pregnancy outcomes. Am J Clin Nutr 2013;97:808-815.

[32] Aldámiz-Echevarría L, Bilbao A, Andrade F, Elorz J, Prieto JA, Rodríguez-Soriano J. Fatty acid deficiency profile in children with food allergy managed with elimination diets. Acta Paediatr 2008;97:1572-1576.

[33] Svennerholm L. Distribution and fatty acid composition of phosphoglycerides in normal human brain. J Lipid Res 1968;9:570-579.

[34] McCann JC, Ames BN. Is docosahexaenoic acid (DHA), an n-3 long chain polyunsaturated fatty acid, required for normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals: a review. Am J Clin Nutr 2005;82:281-295.

[35] Else PL, Hulbert AJ. Membranes as metabolic pacemakers. Clin Exp Pharmacol Physiol 2003;30:559-564.

[36] Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 4th ed. New York, NY, Garland Science 2002.

[37] Meyer BJ, Onyiaodike CC, Brown EA, Jordan F, Murray H, Nibbs RJB. Maternal plasma DHA levels increase prior to 29 days post-LH surge in women undergoing frozen embryo transfer: A prospective, observational study of human pregnancy. J Clin Endocrinol Metab 2016;101(4):1745-1753.

[38] Fliesler SJ, Anderson RE. Chemistry and metabolism of lipids in the vertebrate retina. Prog Lipid Res 1983;2:79-131.

[39] McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development andfunction: Potential implications for the pathogenesis and prevention of psychopathology. Prostaglandins Leukot Essen Fatty Acids 2006;74:329-49.

[40] Giusto NM, Pasquaré SJ, Salvador GA, Castagnet PI, Roque ME, Ilincheta de Boschero MG. Lipid metabolism in vertebrate retinal rod outer segments. Prog Lipid Res 2000;39:315-391.

[41] Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements.Early Hum Dev 1980;4:121-129.

[42] Martinez M. Tissue levels of polyunsaturated fatty acids during early human development.J Pediatr 1992;120:S129-S138.

[43] Hoffman DR, Birch EE, Birch DG, Uauy R, Castaneda Y, Lapus MG *et al.* Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. J Pediatr Gastroenterol Nutr 2000;31:540-553.

[44] Lauritzen L, Hansen HS, Jùrgensen MH, Michaelsen KF. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. Prog Lipid Res 2001;40:1-94.

[45] Carver JD, Benford VJ, Han B, Cantor AB. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. Brain Res Bull 2001;56:79-85.

[46] Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes (Review). Prog Lipid Res 2001;40:439-452.

[47] Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E Prenatal fatty acid status and child adiposity at age 3 y: results from a US pregnancy cohort. Am J Clin Nutr 2011;93(4):780-788. doi: 10.3945/ajcn.110.005801.

[48] Bernard JY, 1, Tint MT, Aris IM, Chen LW, Quah PL, Tan KH *et al*. Maternal plasma phosphatidylcholine polyunsaturated fatty acids during pregnancy and offspring growth and adiposity. Prostaglandins Leukot Essent Fatty Acids 2017; 121:21-29. doi:10.1016/j.plefa.2017.05.006.

[49] Stonehouse W. Does consumption of LC omega-3 PUFA enhance cognitive performance in healthy school-aged children and throughout adulthood? Evidence from clinical trials. Nutrients 2014;6:2730-2758. doi:10.3390/nu6072730.

[50] Weiser MJ, Butt CM, Mohajeri MH. Docosahexaenoic acid and cognition throughout the lifespan. Nutrients 2016;8:99. doi:10.3390/nu8020099.

[51] Helland IB, Smith L, Saarem K, Saugstad OD, Drevon CA. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. Pediatrics 2003;111:e39-e44. URL; http://www.pediatrics.org/cgi/content/full/111/1/e39.

[52] Hibbeln JR, Davis JM, Emmett P, Rogers I, Williams C, Golding J. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet 2007;369:578-585.

[53] Rees A, Sirois S, Wearden A. Maternal docosahexaenoic acid intake levels during pregnancy and infant performance on a novel object search task at 22 months. Child Dev 2014;85:2131-2139.

[54] Drover JR, Hoffman DR, Castañeda YS, Morale SE, Garfield S, Wheaton DH *et al.* Cognitive function in 18-month-old term infants of the DIAMOND study: A randomized, controlled clinical trial with multiple dietary levels of docosahexaenoic acid. Early Human Development 2011;87:223-230.

[55] Colombo J, Jill Shaddy D, Kerling EH, Gustafson KM, Carlson SE. Docosahexaenoic acid (DHA) and arachidonic acid (ARA) balance in developmental outcomes. Prostaglandins Leukot Essent Fatty Acids 2017;121:52-56. doi: 10.1016/j.plefa.2017.05.005.

[56] Hadley K, Ryan A, Forsyth S, Gautier S, Salem N. The essentiality of arachidonic acid in infant development. Nutrients 2016;8:216.

[57] Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implication for cardiovascular prevention. Biochem Pharm 2009;77:937-946.

[58] Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. J Am Diet Assoc 2009;109:668-679.

[59] Ryan AS, Nelson EB. Assessing the effect of docosahexaenoic acid on cognitive functions in healthy, preschool children: a randomized, placebo- controlled, double-blind study. Clin Pediatr 2008;47:355-362.

[60] Dalton A, Wolmarans P, Witthuhn RC, Stuijvenberg ME, Swanevelder SA, Smuts CM. A randomised control trial in schoolchildren showed improvement in cognitive function after consuming a bread spread, containing fish flour from a marine source. Prostaglandins Leukot Essent Fatty Acids 2009;80:143-149.

[61] Dangardt F, Osika W, Chen Y, Nilsson U, Gan LM, Gronowitz E *et al*. Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents. Atherosclerosis 2010;212:580-585.

[62] Hawthorne KM, Abrams SA, Heird WC. Docosahexaenoic acid (DHA) supplementation of orange juice increases plasma phospholipid DHA content of children. J Am Diet Assoc 2009;109:708-712.

[63] The NEMO Study Group. Effect of a 12-mo micronutrient intervention on learning and memoryin well-nourished and marginally nourished school-aged children: 2parallel, randomized, placebo-controlled studies in Australia and Indonesia, Am J Clin Nutr 2007;86:1082-1093.

[64] Muthayya S, Eilander A, Transler C, Thomas T, Knaap H, Srinivasan K *et al*. Effect of fortification with multiple micronutrients and n23 fatty acids on growth and cognitive performance in Indian school children: the CHAMPION (Children's Health and Mental Performance Influenced by Optimal Nutrition) Study. Am J Clin Nutr 2009;89:1766-1775.

[65] Richardson AJ, Burton JR, Sewell RP, Spreckelsen TF, Montgomery P. Docosahexaenoic acid for reading, cognition and behavior in children aged 7-9 years: A randomized, controlled trial (The DOLAB Study). PLOS ONE 2012;7(9):e43909.

[66] Barinaga M. New lead to brain neuron regeneration. Science 1998;282:1018-1019.

[67] Doidge N. The Brain That Changes Itself. New York, Viking Press 2007.

[68] Ahmad A, Murthy M, Greiner RS, Moriguchi T, Salem N Jr. A decrease in cell size accompanies a loss of docosahexaenoate in the rat hippocampus. Nutr Neurosci 2002;5:103-113.

[69] Takeuchi T, Fukumoto Y, Harada E. Influence of a dietary n−3 fatty acid deficiency on the cerebral catecholamine contents, EEG and learning ability in rat. Behav Brain Res 2002;131:193-204.

[70] Ximenes da Silva A, Lavialle F, Gendrot G, Guesnet P, Alessandri JM, Lavialle M. Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. J Neurochem 2002;81:1328-1337.

[71] Nanjo A, Kanazawa A, Sato K, Banno F, Fujimoto K. Depletion of dietary n-3 fatty acid affects the level of cyclic AMP in rat hippocampus. J Nutr Sci Vitaminol 1999;45:633-641.

[72] Gazzah N, Gharib A, Croset M, Bobillier P, Lagarde M, Sarda N. Decrease of brain phospholipid synthesis in free-moving n-3 fatty acid deficient rats. J Neurochem 1995; 64:908-918.

[73] Murthy M, Hamilton J, Greiner RS, Moriguchi T, Salem N, Kim HY. Differential effects of n-3 fatty acid deficiency on phospholipid molecular species composition in the rat hippocampus. J Lipid Res 2002;43:611-617.

[74] Milte CM, Sinn N, Buckley JD, Coates AM, Young R, Howe PRC. Polyunsaturated fatty acids, cognition and literacy in children with ADHD with and without learning difficulties. J Child Health Care 2011;15(4):299-311.

[75] Sinn N, Bryan J. Effect of supplementation with polyunsaturated fatty acids and micronutrients on ADHD-related problems with attention and behaviour. J Developmental & Behavioral Pediatrics 2007;28(2):82-91.

[76] Hibbeln JR, Gow RV. Omega-3 fatty acid and nutrient deficits in adverse neurodevelopment and childhood behaviors Child adolesce. Psychiatr Clin A Am 2014;23(3):555-590. doi:10.1016/j.chc.2014.02.002.

[77] Murakami K, Miyake Y, Sasaki S, Tanaka K, Arakawa M. Fish and *n*-3 Polyunsaturated Fatty Acid Intake and Depressive Symptoms: Ryukyus Child Health Study. Pediatrics 2010;126:e623-e630.

[78] Sichert-Hellert W, Wicher M, Kersting M. Age and time trends in fish consumption pattern of children and adolescents, and consequences for the intake of long-chain n-3 polyunsaturated fatty acids. Eur J Clin Nutr 2009;63:1071-1075

[79] Sioen I, Huybrechts I, Verbeke W, Van Camp J, De Henauw S. n-6 and n-3 PUFA intakes of pre-school children in Flanders, Belgium. Br J Nutr 2007;98:819-825

[80] Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair AJ, Howe PRC. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. Lipids 2003;38:391-398.

[81] Ervin B, Wright JD, Wang CY, Kennedy-Stephenson J, Division of Health and Nutrition Examination Surveys. Dietary Intake of fats and fatty acids for the United States population: 1999–2000. Advanced data from vital health statistic, CDC Number 348: November 8, 2004.

[82] Howe P, Meyer B, Record, Baghurst K. Dietary intake of long-chain ω-3 polyunsaturated fatty acids:contribution of meat sources. Nutrition 2006;22:47-53.

[83] Barbarich BN, Willows ND, Wang L, Clandinin MT. Polyunsaturated fatty acids and anthropometric indices of children in rural China. Eur J ClinNutr 2006;60:1100-1107.

[84] Bermudez OI, Toher C, Montenegro-Bethancourt G, Vossenaar M, Mathias P, Doak C *et al*. Dietary intakes and food sources of fat and fatty acids in Guatemalan school children: A cross-sectional study. J Nutr 2010;9:20.

[85] Yakes EA, Arsenault JE, Islam MM, Ahmed T, German JB, Drake C *et al*. Dietary intake of polyunsaturated fatty acids (PUFA) among breastfeeding and non-breastfeeding 24-48 month old children in Bangladesh. J Pediatr Gastroenterol Nutr 2011;52(3):351-359. doi:10.1097/MPG.0b013e3182042bc8.

[86] Orton HD, Szabo NJ, Clare-Salzler M *et al.* Comparison between omega-3 and omega-6 polyunsaturated fatty acid intakes as assessed by a food frequency questionnaire and erythrocyte membrane fatty acid composition in young children. Eur J ClinNutr 2008;62:733-738.

[87] EFSA Panel on Dietetic Products Nutrition and Allergies (NDA). 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;8:1461.

[88] AFFSA (France). Avis de l'agence francaise de securite sanitaire des ailments relatif a l'actualisation des apports nutritionels conseilles pour les acides gras. http://www.afssa.fr/cgi-bin/countdocs.cgi?Documens/NUT2006sa0359EN.pdf (24 April 2018),

[89] Health Council of the Netherlands. Guidelines for a healthy diet 2006.

[90] Nordic Nutrition Recommendations (NNR). Integrating nutrition and physical activity; Copenhagen, 2004.

[91] Meyer BJ. Australians are not meeting the recommended intakes for omega-3 long chain polyunsaturated fatty acids: Results of an analysis from the 2011-2012 national nutrition and physical activity survey. Nutrients 2016;8:111.

[92] Cosatto VF, Else PL, Meyer BJ. Do pregnant women and those at risk of developing post-natal depression consume lower amounts of long chain omega-3 polyunsaturated fatty acids? Nutrients 2010;2:198-213.

[93] Donahue SM, Rifas-Shiman SL, Olsen SF, Gold DR, Gillman MW, Oken E. Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. Prostaglandins Leukot Essent Fatty Acids 2009;80:289-296.

[94] Zhang Z, Fulgoni VL, Kris-Etherton PM, Mitmesser SH. Dietary intakes of epa and dha omega-3 fatty acids among us childbearing-age and pregnant women: An analysis of nhanes 2001-2014. Nutrients 2018;10.

[95] Ueshima H, Stamler J, Elliott P, Chan Q, Brown IJ, Carnethon MR *et al.* Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: Intermap study. Hypertension 2007;50:313-319.

[96] Kobayashi M, Sasaki S, Kawabata T, Hasegawa K, Akabane M, Tsugane S. Single measurement of serum phospholipid fatty acid as a biomarker of specific fatty acid intake in middle-aged japanese men. Eur J Clin Nutr 2001;55:643-650.

[97] Kim YS, Xun P, Iribarren C, Van Horn L, Steffen L, Daviglus ML *et al.* Intake of fish and long-chain omega-3 polyunsaturated fatty acids and incidence of metabolic

syndrome among american young adults: A 25-year follow-up study. Eur J Nutr 2016;55:1707-1716.

98 Emmett R, Akkersdyk S, Yeatman H, Meyer BJ. Expanding awareness of docosahexaenoic acid during pregnancy. Nutrients 2013;5:1098-1109.

99 International Society for the Study of Fatty Acids and Lipids (ISSFAL). http://www.issfal.org/pufa-recommendations (28 April 2018)

100 Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I *et al.* The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: Review of current knowledge and consensus statement. J Perinatal Medicine 2008;36:5-14.

101 D-A-C-H. *Deutsche gesellschaft fur ernahrung, osterreichische gesellschaft fur ernahrung schweizerische gesellschaft fur ernahrungsforschung, schweizererische vereinigung fur ernahrung: Referenzwerte fur die nahrstoffzufuhr, umschau/braus verlag, frankfurt* 2008.

102 Superior Health Council of Belgium. Recommendations and claims made on omega-3 fatty acids (shc7945). https://www.health.belgium.be/en/recommendations-and-claims-made-omega-3-fatty-acids-2004-shc-7945 (3 May 2018)

103 Koletzko B, Cetin I, Brenna JT, Perinatal Lipid Intake Working G, Child Health F, Diabetic Pregnancy Study G et al. Dietary fat intakes for pregnant and lactating women. Br J Nutr 2007;98:873-877.

104 March of Dimes. Omega-3 fatty acids during pregnancy. https://www.marchofdimes.org/pregnancy/vitamins-and-other-nutrients-during-pregnancy.aspx (30 April 2018)

105 Micha R, Khatibzadeh S, Shi P, Fahimi S, Lim S, Andrews KG et al. Global, regional, and national consumption levels of dietary fats and oils in 1990 and 2010: a systematic analysis including 266 country-specific nutrition surveys. BMJ 2014;348:g2272. doi: 10.1136/bmj.g2272.

106 Innis SM. Dietary omega-3 fatty acids and the developing brain. Brain Research 2008;1237:35-43

107 Mietus-Snyder M, Krauss RM. Lipid metabolism in children and adolescents: impact on vascular biology. J Clin Lipidol 2008;2:127-137.

108 Niinikoski H, Lagström H, Jokinen E, Siltala M, Rönnemaa T, Viikari J *et al*. Impact of repeated dietary counselling between infancy and 14 years of age on dietary intakes and serum lipids and lipoproteins: the STRIP study. Circulation 2007;116:1032-1040.

109 Moyad MA. An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: Part I. Urologic Oncology 2005;23:28-35.

[110] Moyad MA. An introduction to dietary/supplemental omega-3 fatty acids for general health and prevention: Part II. Urologic Oncologic 2005;23:36-48.

[111] Dangardt F, Osika W, Chen Y, Nilsson U, Gan LM, Gronowitz E *et al*. Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents. Atherosclerosis 2010;212:580-585.

[112] Hawthorne KM, Abrams SA, Heird WC. Docosahexaenoic acid (DHA) supplementation of orange juice increases plasma phospholipid DHA content of children. J Am Diet Assoc 2009;109:708-712.

[113] López-Huertas E. Health effects of oleic acid and long chain omega-3 fatty acids (EPA and DHA) enriched milks. A review of intervention studies, Pharmacological Res 2010;61:200-207.

[114] Ridker PM. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. Circulation 2003;107:363-369.

[115] Klein-Platat C, Drai J, Oujaa M, Schlienger J, Simon C. Plasma fatty acid composition is associated with the metabolic syndrome and low-grade inflammation in overweight adolescents. Am J Clin Nutr 2005;82:1178-1184.

[116] Harris WS, Von Schacky C. The omega-3 index: a new risk factor death from coronary heart disease? Prev Med 2004;39:212-220.

[117] Burrows T, Collins CE, Garg ML. Omega-3 index, obesity and insulin resistance in children. Int J Pediatr Obes 2011;6:e532-e539.

[118] Harris WS. The omega-3 index as a risk factor for coronary heart disease. Am J Clin Nutr 2008;87(suppl):1997S-2002S.

[119] von Schacky C. The omega-3 index as a risk factor for cardiovascular diseases. Prostaglandins Other Lipid Mediat 2011;96:94-98.

[120] Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004;255:188-205.

[121] O'Sullivan TA, Ambrosini GL, Mori TA, Beilin LJ, Oddy WH. Omega-3 index correlates with healthier food consumption in adolescents and with reduced cardiovascular disease risk factors in adolescent boys. Lipids 2011;46:59-67.

[122] Saito E, Okada T, Abe Y, Kuromori Y, Miyashita M, Iwata F *et al*. Docosahexaenoic acid content in plasma phospholipids and desaturase indices in obese children. J Atheroscler Thromb 2011;18:345-350.

[123] Romeo J, Wärnberg J, García-Mármol E, Rodrı´guez-Rodrı´guez M, Diaz LE, Gomez-Martı´nez S *et al.* Daily consumption of milk enriched with fish oil, oleic acid, minerals and vitamins reduces cell adhesion molecules in healthy children. Nutr Metab Cardiovasc Dis 2011;21:113-120.

[124] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993;362:801-809.

[125] Roldán V, Marín F, Lip GYH, Blann AD. Soluble E-selectin in cardiovascular disease and its risk factors: a review of the literature. Thromb Haemost 2003;90:1007-1020.

[126] Engler MM, Engler MB, Malloy M, Chiu E, Besio D, Paul S *et al.* Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY Study. Int J Clin Pharmacol Ther 2004;42:672-679.

[127] Mardia L, Araceli M-C, Oscar V-C, Enrique R-M, José F. Supplementation of n3 long-chain polyunsaturated fatty acid synergistically decreases insulin resistance with weight loss of obese prepubertal and pubertal children. Archives of Medical Research 2011;42:502-508.

[128] Burns-Whitmore BL, Haddad EH, Sabate J *et al*. Effect of n-3 fatty acid enriched eggs and organic eggs on serum lutein in free-living lacto-ovo vegetarians. Eur J ClinNutr 2010;64:1332-1337.

[129] Li D, Sinclair A, Mann N, Turner A, Kelly F, Abedin L. Effect of dietary α-linolenic acid on thrombotic risk factors in vegetarian men. Am J Clin Nutr 1999;69:872-882.

[130] SACN/COT Scientific Advisory Committee on Nutrition (SACN) and Committee on Toxicity (COT) authors (2004) Advice on Fish Consumption: Benefits and Risks. TSO, Norwich, UK.

[131] Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ, Holub BJ. Effect of dietary _-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. Lipids 1993;28: 811-817.

[132] Murphy KJ, Meyer BJ, Mori TA, Burke V, Mansour J, Patch CS *et al.* Impact of food enriched with n-3 long-chain polyunsaturated fatty acids on erythrocyte n-3 levels and cardiovascular risk factors. Br J Nutr 2007;97:749-757.

[133] Gillingham LG, Caston L, Leeson S, Hourtovenko K, Holub BJ. The effects of consuming docosahexaenoic acid (DHA)-enriched eggs on serum lipids and fatty acid composition in statin-treated hypercholesterolemic male patients. Food Res International 2005;38:1117-1123.

[134] Mu H. Bioavailability of omega-3 long-chain polyunsaturated fatty acids from foods. Agro Food Industry hi-tech 2008;19:24-26.

[135] Kolanowski W. Bioavailability of omega-3 PUFA from foods enriched with fish oil-A mini review. Pol J Food Nutr Sci 2005;14:335-340.

[136] Newton I, Snyder D. Nutritional aspects of long-chain omega-3 fatty acids and their use in bread enrichment. Cereal Foods World 1993;3:126-131. Available at: http://www.ceresconsulting.com/images/BREAD_Fortification.pdf, accessed on June, 13, 2012.

[137] Wallace JMW, McCabe AJ, Robson PJ, Keogh MK, Murray CA, Kelly PM *et al*. Bioavailability of n-3 polyunsaturated fatty acids (PUFA) in food enriched with microencapsulated fish oil. Ann Nutr Metab 2000;44:157-162.

[138] Liu M, Wallin R, Saldeen T. Effect of bread containing stable fish oil on plasma phospholipid fatty acids, triglycerides, HDL-cholesterol and malondialdehyde in subjects with hyperlipidemia. Nutr Res 2001;21:1403-1410.

[139] Church DF, Pryor WA. Free radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 1985;64:111-126.

[140] Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. Clinical Chemistry 1997;43:1209-1214.

[141] Yep YL, Li D, Mann NJ, Bode O, Sinclair AJ. Bread enriched with microencapsulated tuna oil increases plasma docosahexaenaoic acid and total omega-3 fatty acids in humans. Asia Pac J Clin Nutr 2002;11:285-291.

[142] Grune T, Kramer K, Hoppe PP, Siems W. Enrichment of eggs with n-3 polyunsaturated fatty acids: effects of vitamin E supplementation. Lipids 2001;36:833-838.

[143] Lopez-Ferrer S, Baucells MD, Barroeta AC, Grashorn MA. n-3 enrichment of chicken meat. 1. Use of very long-chain fatty acids in chicken diets and their influence on meat quality: fish oil. Poult Sci 2001;80:741-752.

[144] Ayerza R, Coates W. Dietary Levels of Chia: Influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. Poultry Science 2000;79:724-739.

[145] Jiang Z, Sim JS. Consumption of n-3 polyunsaturated fatty acid-enriched eggs and changes in plasma lipids of human subjects. Nutrition 1993;9:513-518.

[146] Ferrier LK, CastonLJ, Leeson S, Squires J, Weaver BJ, Holub BJ. α-Linolenic acid- and docosahexaenoic acid-enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipid fatty acids in humans. Am J Clin Nutr 1995;62:81-86.

[147] Delaroudis S, Slavakis A, Kyroudi A, Tzoiti, Sarris, Balaris et al. Omega-3 PUFA modified eggs: a study on their effect on the lipid profile of human volunteers. 71st EAS' Congress and Satellite Symposia 1999;130-131.

[148] Lewis NM, K Schalch, Scheideler. Serum lipid response to n-3 fatty acid enriched eggs in persons with hypercholesterolemia. J Am Diet Assoc 2000;100:365-367.

[149] Gillingham LG, Caston L, Leeson S, Hourtovenko K, Holub BJ. The effects of consuming docosahexaenoic acid (DHA)-enriched eggs on serum lipids and fatty acid composition in statin-treated hypercholesterolemic male patients. Food Res International 2005;38:1117-1123.

[150] Öhman M, Åkerfeldt T, Nilsson I, Rosen C, Hansson LO, Carlsson M *et al*. Biochemical effects of consumption of eggs containing omega-3 polyunsaturated fatty acids. Upsala J Med Sci 2008;113:315-323.

[151] Walldius G, Jungner I. Apolipoprotein B and apolipoprotein A-I: risk indicators of coronary heart disease and targets for lipid-modifying therapy. J Intern Med 2004;255:188-205.

[152] Savas Erdeve S, Simsek E, Dallar Y, Biyikli Z. Utility of ApoB/ApoA1 ratio for the prediction of cardiovascular risk in children with metabolic syndrome. Indian J Pediatr 2010;77:1261-1265.

[153] Nolan JM, Stack J, O'Connell E, Beatty S. The relation between macular pigment optical density and its constituent carotenoids in diet and serum. Invest Ophthalmol Vis Sci 2007;48:571-582.

[154] Surai P, MacPherson A, Speake BK, Sparks NH. Designer egg evaluation in a controlled trial. Eur J Clin Nutr 2000;54:298-305.

[155] Burns-Whitmore BL, Haddad EH, Sabate J, Jaceldo-Siegl K, Tanzman J, Rajaram S. Effect of n-3 fatty acid enriched eggs and organic eggs on serum lutein in free-living lacto-ovo vegetarians. Eur J Clin Nutr 2010;64:1332-1337.

[156] Johnson EJ, Chung H-Y, Caldarella SM, Snodderly DM. The influence of supplemental lutein and docosahexaenoic acid on serum, lipoproteins, and macular pigmentation. Am J Clin Nutr 2008;87:1521-1529.

[157] Visioli F, Rise P, Plasmati E, Pazzucconi F, Sirtori CR, Galli C. Very low intakes of n-3 fatty acids incorporated into bovine milk reduce plasma triacylglycerol and increase HDL-cholesterol concentrations in healthy subjects. Pharm Res 2000;41:571-576.

[158] Higgins S, Carroll YL, O'Brien NM, Morrissey A. Use of microencapsulated fish oil as a mean of increasing n-3 polyunsaturated fatty acid intake. J Hum Nutr Diet 1999;12:265-271.

[159] Baró L, Fonollá J, Peńa JL, Martínez-Férez A, Lucena A, Jiménez J *et al.* n-3 fatty acids plus oleic acid and vitamin supplemented milk consumption reduces total and LDL cholesterol, hemocysteine and level of endothelial adhesion molecules in healthy humans. Clin Nutr 2003;22:175-182.

[160] Ohta T, Saku K, Takata K, Adachi N. Soluble vascular cell-adhesion molecule-1 and soluble intercellular adhesion molecule-1 correlate with lipid and apolipoprotein risk factors for coronary artery disease in children. Eur J Pediatr 1999;158:592-598.

[161] Blankenberg S, Rupprecht HJ, Bickel C, Jiang XC, Poirier O, Lackner KJ *et al.* Common genetic variation of the cholesteryl ester transfer protein gene strongly predicts future cardiovascular death in patients with coronary artery disease. J Am Coll Cardiol 2003;41:1983-1989.

[162] Thienprasert A, Samuhaseneetoo S, Popplestone K, West AL, Miles EA, Calder PC. Fish oil n-3 polyunsaturated fatty acids selectively affect plasma cytokines and decrease illness in Thai school children: A randomized, double-blind, placebo-controlled intervention trial. J Pediatrics 2009;154:391-395.

[163] Jacobsen C, Let MB, Andersen G, Meyer AS. Oxidative stability of fish oil enriched yoghurts. In Seafood research from fish to dish: Ouality, safety and processing of wild and farmed fish, 1st ed. Luten JB, Jaconsen C, Bakaert K, Sæbø A, Oehlenschläger J, Eds. Wageningen Academic Publishers: Wageningen, The Nederlands 2006.

[164] Let MB, Jacobsen C, Meyer. Lipid oxidation in milk, yoghurt, and salad dressingenriched with neat fish oil or pre-emulsified fish oil. J Agric Food Chem 2007;55:7802-7809.

[165] Farvin KH, Baron CP, Nielsen NS, Jacobsen C. Antioxidant activity of yoghurt peptides: Part 1-in vitro assays and evaluation in ω-3 enriched milk. Food Chemistry 2010;123:1081-1089.

[166] Schram LB, Nielsen CJ, Porsgaard T, Nielsen NS, Holm R, Mu H. Food matrices affect the bioavailability of (n-3) polyunsaturated fatty acids in a single meal study in humans. Food Res Inter 2007;40:1062-1068.

[167] Garg ML, Blake RJ, Clayton E, Munro IA, Macdonald-Wicks L, Singh H *et al.* Consumption of an n-3 polyunsaturated fatty acid-enriched dip modulates plasma lipid profile in subjects with diabetes type II. Eur J Clin Nutr 2007;61:1312-1317.

[168] Arterburn LM, Oken HA, Bailey Hall E, Hamersley J, Kuratko CN, Hoffman JP. Algal-oil capsules and cooked salmon: nutritionally equivalent sources of docosahexaenoic acid. J Am Diet Assoc 2008;108:1204-1209.