Maternal docosahexaenoic acid status during pregnancy and its impact on infant neurodevelopment

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Abstract

Dietary components are important for the structural and functional development of the brain. Among these, docosahexaenoic acid,22:6n-3 (DHA) is critically required for the structure and development of the growing fetal brain *in utero*. DHA is the major n-3 long-chain fatty acid in brain gray matter representing about 15% of all fatty acids in the human frontal cortex. DHA affects neurogenesis, neurotransmitter, synaptic plasticity & transmission, and signal transduction in the brain. Studies in animals and humans show that adequate levels of DHA in neural membranes are important for cortical astrocyte maturation and vascular coupling, and helps cortical glucose uptake and metabolism. In addition, specific metabolites of DHA are bioactive molecules that protect tissues from oxidative injury and stress in the brain. A low DHA level in the brain results in behavior changes and is associated with learning problems and memory deficits. In humans, the third trimester-placental supply of maternal DHA to the growing fetus is critically important as the growing brain obligatory requires DHA during this window period. Besides, DHA is also involved in the early placentation process, essential for placental development. This underscores the critical importance of maternal DHA intake for the structural and functional development of the brain. This review describes DHA's multiple roles during gestation, lactation, and the consequences of its lower intake during pregnancy and postnatally on the children's brain development and function.

Keywords: DHA, Brain, MFSD2a, SPM, Fetus, Placenta, infacnts, Neurogenesis, Pregnancy, Pre-term

Abbreviations used: DHA ,Docosahexaenoic acid, 22:6 (n-3); EPA, Eicosapentaenoic acid, 20:5 (n-3); ARA, Arachidonic acid, 20:4 (n-6); ALA, Alpha-linolenic acid, 18:3 (n-3); LA: LA, Linoleic acid, 18:2 (n-6); Long chain polyunsaturated fatty acids, LCPUFAs; Resolvins, Rvs; Protectin D_1, PD1; G protein-coupled receptor, GPR; Specialized proresolving mediators, SPMs:

Introduction

Docosahexaenoic acid, 22:6n-3(DHA), a member of the n-3 long-chain polyunsaturated fatty acids (LCPUFAs), plays several important roles in human health [1-4]. DHA modulates processes such as inflammation, angiogenesis, cell proliferation, signal transduction, membrane structure and function, and a host of other cellular and molecular functions affecting health and diseases [3,5,6]. Several metabolites of DHA also play roles in biochemical and cellular processes[7]. The low intake of dietary DHA and/or its precursor, eicosapentaenoic acid, 22:5n-3 (EPA) is responsible for poor feto-placental growth and development, risk of cardiovascular disease, inflammatory disorders, mental stress, behavioural changes and poor cognitive features[5,8]. While EPA and DHA are both utilized for cell signaling molecules, eicosanoids, and docosanoids, DHA is mainly used for membrane structure and function [3,4]. Modern refined diets are mostly deficient in the n-3 long-chain polyunsaturated fatty acids (LCPUFAs), leading to sub-optimal organ function that may predispose individuals to an increased risk of disease. Several experts made recommendations for the increase in n-3 LCPUFAs intake either from an increased seafood intake or via supplements. Recommendations for EPA and DHA intakes have been reported based on data supporting health outcomes for heart disease and cognitive development. A minimum daily intake of 250–500 mg of n-3 LCPUFAs for healthy adults appears to be the general consensus among the scientific community. Few countries have proper guidelines for the consumption of n-3 fatty acids. DHA intake during pregnancy is an important consideration as it is involved in fetal-brain's growth and development. DHA also contributes to early placentation processes, as the recent findings suggest the DHA requirement as an important critical step for placental growth and development. Low DHA intakes at early stages in pregnancy may lead to incomplete placentation and can affect feto-placental growth. Therefore, it is urgent to consider DHA's supplementation well before the gestation to prevent placenta-related disorders. There are

numerous reviews available about DHA on human health[3,4,9-11]. However, this review focuses mainly on the roles of maternal DHA during pregnancy on neurocognitive development in infants.

DHA and its metabolites: effects on the structure and function of the human brain

N-3 PUFAs have essential functions on human health, and various studies have explained the molecular mechanisms underlying the effects of DHA in various tissues, including the brain. DHA is the predominant n-3 LCPUFA within the brain. Several neurophysiological functions are attributed to DHA, including the regulation of cell-survival, neuroinflammation, and neurogenesis and signal transduction, as well as a newly identified role in blood-brain barrier permeability. Given the proposed universal roles of DHA in the brain, it may come as no surprise that dysregulation of brain DHA metabolism has been implicated in several neurological and psychiatric conditions, whether as a cause or consequence is not agreed upon. Various derivatives of DHA are known to have diverse biological activities. This superfamily of specialized proresolving mediators (SPMs) include: lipoxins, resolvins [resolvin D (RvD) and resolvin E (RvE)], protectins, and maresins[12,13]. SPMs actively turn off the inflammatory response by acting on distinct G-protein-coupled receptors (GPCR) expressed on immune cells that activate dual anti-inflammatory and proresolution processes [12,13]. Among the anti-inflammatory actions of SPMs include the induction in the expression of anti-inflammatory cytokines or inflammatory scavenging molecules such as IL-10, IL-1 decoy receptors, and IL-1 receptor antagonists[12,13]. Conversely, SPMs activate specific mechanisms that trigger the resolution of inflammation, including downregulation of pro-inflammatory cytokines, abrogation of intracellular pathways that lead to inflammation, clearance of inflammatory cell debris by macrophages, and normalization of immune cells counts to basal levels [12,13]. The importance of SPMs in the resolution of inflammation is evidenced in several chronic pathological conditions including brain inflammation in which their production remained insufficient, delayed, or even absent, and exogenous administration

of SPMs reduces inflammation and protects inflamed tissue[12,14]. Dietary supplementation with metabolic precursors of SPMs can increase their availability and thus resolve inflammation after neurological injury. In the brain, DHA and ARA, the fatty acid metabolic precursors, are incorporated into cell membranes and thus influence their metabolism. N-6 derived lipid mediators, including prostaglandin E2 and leukotriene B4, may prolong inflammation whereas those of n-3 fatty acids decrease these inflammatory compounds and their activity. Thus, with n-3 fatty acids supplementation, the balance of lipid mediator metabolic precursors can be restored or reversed by providing substrates for SPM production. Metabolic precursors of the inflammation-resolving SPMs, including DHA, have potent anti-inflammatory effects in traumatic brain injury [15]. The low and medium doses, but not a high dose of DHA, resulted in significant tissue sparing in the peri-infarct region in middle cerebral artery occlusion rats when treated intravenously with high (70 mg/kg bw), medium (16 or 35 mg/kg), or low (3.5 mg/kg) doses of DHA [16]. Treated rats showed significantly better performance in neurological function up to 7 days following middle cerebral artery occlusion. Following experimental traumatic brain injury in rats, a DHA-enriched diet for 12 days preserved otherwise depleted brain-derived neurotrophic factor (BDNF) and also improved learning performance[15]. While DHA's mechanism of action in rescuing neurological injury remain unknown, its therapeutic potential is compelling for further investigations. Resolvins, protectins, and maresins have SPM functions. These are generated from DHA either spontaneously or in the presence of aspirin by lipoxygenase and acetylated cyclooxygenase-2 (COX-2). DHA is the substrate for two groups of the resolvins (Rvs) produced by different biosynthetic routes, referred to as 17S- and 17RD- series resolvins during the resolution of inflammatory exudates [17,18]. Rvs have potent anti-inflammatory and pro-resolution actions in animals[7]. Rv of D and E series are endogenous lipid mediators generated from DHA and EPA. Each of RvE1 and RvD1 can potentially decrease inflammatory status. DHA can be converted to 17(S)-hydroxy-containing RvD1–D4 and conjugated triene-containing docosanoid structures through lipoxygenase (LOX) [19]. Rvs normalize the exaggerated pain

via regulation of inflammatory mediators, transient receptor potential ion channels, and spinal cord synaptic transmission. RvD_1 is 17(S)-trihydroxy-docosahexaenoic acid and structurally differs from aspirin-triggered RvD_1, 17(R)-trihydroxy-docosahexaenoic acid, produced by the action of COX-2 followed by the lipoxygenase action on 13-hydroxyDHA[20]. RvD_1 blocks the transcription of IL-1β that prevents neutrophil (PMN)infiltration into the brain by its anti-inflammatory action[18]. The Rvs characterized so far are all potent anti-inflammatory compounds, therefore, the overall extent of Rv synthesis from n-3 LCPUFAs might constitute an important parameter for the assessment of the beneficial effects of n-3 PUFA supplementation.

Protectin D_1 (PD1), an endogenously produced anti-inflammatory compound from DHA in neural tissues and hence, named as neuroprotectin (NPD_1). The endogenously produced NPD_1; 17(S)-trihydroxy DHA is structurally different from aspirin-triggered NPD_1; 17(R)-trihydroxy DHA. Protectins have an action against bacterial and viral infections. PD_1 exerts anti-inflammatory activity by controlling PMN infiltration both *in vitro* and *in vivo*. NPD_1 is effective in preventing damage to retina, brain, liver, and kidney and may avert fibrosis in mice[21]. PDX is an isomer of PD1/NPD1, which is known to have anti-inflammatory and anti-atherogenic properties[22,23]. In addition to showing the insulin-sensitizing and glucose regulatory actions, PDX can also suppress lipid-induced inflammation, possibly via IL-6 secretion[24,25]. Maresins (macrophage mediators in resolving inflammation) are a genus of resolution agonists biosynthesized from DHA by macrophages that display anti-inflammatory and pro-resolving actions[26]. Maresin 1 (MaR1; 7*R*,14*S*-dihydroxy-docosa-4*Z*,8*E*,10*E*,12*Z*,16*Z*,19*Z*-hexaenoic acid) is a family of structurally distinct autacoids [27]. Endogenous DHA is metabolized by activated macrophages through 12-LOX followed by soluble epoxy hydrolase activity to form maresins (Mars). There are two known structural

variants of Mars, Mar 1 and Mar 2. Studies on Mar 1 revealed it has potent anti-inflammatory activity even at nano molar range [28].

Resolvins and protectins seem to have similar and overlapping anti-inflammatory actions. It is likely that the generation of 18R E-series resolvins (RvE1 and RvE2) from EPA and 17S D-series resolvins (RvD1to D6), PD1 and Mar from DHA are generated during the resolution phase of acute inflammation. The generation of these anti-inflammatory compounds is an important event in the resolution of inflammation that is essential for the healing process. These anti-inflammatory compounds formed from EPA and DHA inhibit PMN transendothelial migration, reduce leukocyte infiltration, and suppress dendritic cell migration, and IL-12 production needed for anti-inflammation initiatives [29]. The PD1 inhibits the production of IFN-γ and TNF-α by activated T cells and also induces T cell apoptosis which indicates its role in the down-regulation of Th1-mediated responses. It is interesting to note that PD1 is produced by Th2-skewed human peripheral blood mononuclear cells through a LOX-dependent mechanism, supporting that DHA may favor the development of a Th2 phenotype[30] during inflammation that may account for its anti-inflammatory responses. It is evident from these findings that EPA ,DHA and their anti-inflammatory mediators such as Rvs, PDs, and Mars play a significant role in mitigating inflammation that suggests that these bioactive lipids could be exploited as potential drugs for preventing and managing several inflammatory diseases[29].

DHA accretion, supplementation, and fetal brain development

DHA is essential for normal development of a healthy brain [9,31,32]. In fact, DHA is quantitatively the major fatty acid in the brain [33]. EPA level is 250–300 times lower than DHA in human brain tissue. There are also differences in their phospholipid distributions of n-3 LCPUFAs in the brain. DHA is predominantly enriched in phosphatidylethanolamine and phosphatidylserine fractions, whereas EPA is mostly present in phosphatidylinositol fraction.

DHA is the most abundant n-3 LCPUFA in the central and peripheral nervous system, representing the major proportion of PUFAs in the brain and retina. This fatty acid is present in large amounts in phospholipids of brain gray matter[34] . DHA performs an important role in neurogenesis and synaptogenesis, particularly in fetal development and during the first two years of life[35]. Fetal DHA accretion occurs actively along with pregnancy but is most active during the third trimester, as demonstrated after the supplementation of pregnant women with fish oil (200 mg DHA/day). Further, it was observed that DHA supplementation during pregnancy lowered the decline in maternal DHA status during the last trimester[36]. Due to this reason, the nutritional status of DHA during pre-conception, pregnancy, and lactation of the mother represents a critical step for the brain and visual development of her child [37,38]. Neonates with higher DHA concentrations in umbilical plasma phospholipids have a longer gestational length compared to neonates with low concentration[39]. The pregnant women who received 600 mg DHA/day before 20 weeks of gestation demonstrated a significant reduction of preterm delivery and low-weight birth with good tolerance to supplementation and no adverse effects[40]. DHA supplementation improves the nutritional status of the long-chain fatty acids both in the mother and her child because of efficient transfer of these fatty acids through the placenta[41], by the increase of DHA levels in maternal milk [42] and in the phospholipids of umbilical cord blood during lactation[43]. The supplementation of DHA was found beneficial for mothers having low ingestion of marine foods[44]. DHA supplementation during pregnancy increases the transport of n-3 LCPUFA through the placenta by improved expression of fatty acid transport proteins [45,46]. The pregnant women in Australia who consumed fish oil rich in DHA (2.2g DHA/day) and EPA (1.1g EPA/day), from the 20th week of pregnancy until the partum showed improved visual and coordination capacity of the children than olive oil [47]. Similar beneficial effects were obtained after the supplementation

of mothers with 500 mg DHA/day along with the pregnancy, which correlated the high blood DHA levels with improved cognitive development of 5.5 year-old children [48]. Similarly, daily supplements of fish oil (500mg DHA + 150mg EPA) along with 5-methyltetrahydrofolate (400 µg/day)showed a prolonged cognitive activities until 6.5 year-age [49]. Higher DHA in plasma and breast milk, positively correlate the growth and development of the brain and visual acuity in the children [50,51]. These findings have corroborated that supplementing a mother's diet with DHA during pregnancy and lactation, or consumption of formula enriched in DHA, helps to increase the tissue levels of DHA in the infant with a better visual and neurological development[52]. Conversely, during pregnancy and/or lactation, a diet low in n-3 LCPUFA may have direct implications for the child's visual and neurological development [53,54]. An example of this effect is that infants fed breast milk poor in DHA (less than 0.17% of total fatty acids, usually woman milk contains 0.3–0.4% DHA) show lower DHA levels in erythrocytes, reduced visual acuity, and reduced language development at 14 months post-partum compared to infants fed breast milk containing 0.36% DHA[38,55]. A study in pregnant women who received supplementation of DHA (600mg/day) from <20 week of pregnancy until the delivery, showed a significant increase in visual acuity, particularly in newborns males, suggest that DHA supplementation is possibly the best predictor for nervous system development[56]. Several studies have established a direct relationship between higher erythrocyte DHA levels (in mother and children) and the optimal visual and neuronal development of children[57-59], which in the long-term, has benefits in the development of cognitive and motor skills in these children[60] . Even perinatal supplementation with DHA reduced the risk of lower IQ scores in children from families with very low incomes[61,62]. DHA provided to newborn through formulas improves cortical maturation and visual function[63]. A study carried out with six-month-old babies who did not

receive maternal milk and who were fed a formula containing egg yolk enriched with DHA (115mg DHA/100 g food), showed a significant increase in erythrocyte DHA phospholipids and a better visual development as evidenced by improved maturation of retinal and visual cortex of one year infant[64]. The food formulas that supply a minimum of 0.35% DHA favor improved brain development until 4 month after delivery as evaluated by the mental development index at term.[65]. In addition to DHA's dietary intake, arachidonic acid, 20:4n-6 (ARA) is obligatory for optimal brain and eye development in infants[66]. The child who did not receive maternal milk, must be fed with formula, containing DHA and ARA, to avoid a lower brain growth and development, until 39 months after birth,[67] and even 4 years after birth [66]. A study in newborns (n = 343) of 1–9 day-old fed with formula food of varied levels of DHA (DHA 0%; 0.32%; 0.64%; 0.96%, with ARA 0.64% in all formulas) until 12th month, demonstrated that formulas containing DHA (0.32%) produced a better cognitive development compared to control group (0% DHA)[68]. A significant higher capacity of memory and problem-solving skills were observed when preterm child with a birth weight < 1500 g were fed with human milk supplemented in DHA (32 mg/day) and ARA (31 mg/day) until discharge from the hospital i.e., 9 weeks after birth [69]. Higher mental development index scores were also reported from preterm child with a birth weight over 2000 g who received formula enriched in DHA (0.05 g/100 g) and ARA (0.1 g/100 g) [70]. Another study demonstrated that the supplementation of 420 full-term newborn children with n-3 LCPUFA (250 mg of DHA and 60 mg of EPA daily) from their birth until six months, showed a significant accretion in the DHA content of erythrocyte phospholipids and an early development of language and communication skills[71]. A follow-up study of preterm children (n = 107) who received non supplemented and supplemented formula with 0.5% DHA from their birth until nine months demonstrated that girls showed significant benefits in their capacity of alphabetization, verbal

and total intellectual coefficient, and high scores in memory trials[72]. A comparative study among the infants fed on breast milk, a preterm formula supplemented with LCPUFAs, or a traditional preterm formula without LCPUFAs, showed preterm formulas with LCPUFAs can positively improve the visual acuity and development of infants like those fed with maternal milk[73]. A study involving 28 countries found that the levels of DHA in breast milk contribute significantly to achieve better mathematics test scores in children from low-income families. The performance of breastfed children was found superior to non-breastfed children from high-income families and/or increased spending on education [74].

Circulating DHA is significantly related to cognitive abilities during aging and inversely associated with cognitive decline. LCPUFAs serve as an agonist for the GPR40 receptor. GPR40 activation is involved in adult neurogenesis and the concomitant synaptic plasticity. Abnormal activation of GPR40 is thought to cause lipotoxicity in brain endothelial cells and neurons[75]. Many studies have shown that LCPUFAs and GPR40 signaling contributes to the neurogenesis, anti-nociceptive effects, anti-apoptotic effect, Ca^{2+} homeostasis in Alzheimer's disease (AD) and in the functioning of nigrostriatal pathways. GPR40 is now considered as a potential target in the management of several neuropathological conditions. The DHA availability during brain development is also influenced by genetic polymorphisms of enzymes responsible for the endogenous conversion to LCPUFAs from their precursors. It has been shown that the presence of certain polymorphisms in genes encoding Δ-5 and Δ-6 desaturases, enzymes responsible for the formation of n-3 LCPUFA from the precursor ALA, is associated with altered levels of these fatty acids, particularly DHA[76]. For example, the presence of rs 174575 polymorphism in the gene encoding Δ-6 desaturase enzyme in children allows higher tissue concentration of DHA and higher scores on IQ tests[77], a situation that would indicate the importance of gene variations in the metabolism of n-3 LCPUFA and a consequent

beneficial effect on brain development. Children of 9 month-old supplemented with n-3 LCPUFA with high DHA content (supplied from fish oil) showed a significant cognitive performance when they were subjected to "free-play-tests" (evaluation of attention) and better arterial pressure at the end of infancy. These data indicate that n-3 LCPUFA consumption, especially DHA, may positively influence brain development that also has a protective effect against cognitive and cardiovascular diseases in adult life[78]. Pre-school 4 year-old children supplemented with DHA (400 mg) daily for 4 months have significantly raised blood DHA levels, which were positively correlated with increment of punctuation score obtained for vocabulary and comprehension tests[79] . Autochthonous Australian children (n = 409) from 3 to 13 year-old daily supplemented with 750 mg DHA and 60 mg EPA for a period of 20 weeks, exhibited a significant increment of the scholar performance, especially those children between 7 and 12-year-old[80]. The supplementation of fish oil rich in DHA to 7–12-year-old Australian ADHD children showed improved word reading, spelling capacity, and parents' conduct. These changes were positively associated with an increase in the DHA level of erythrocyte phospholipids [81]. Despite these evidences, absolute compliance with DHA supplementation of baby food has not been established yet. The *Scientific Opinion on the essential composition of infant and follow-on formulae, 2014,* of the European Food Safety Authority (EFSA) Panel notes that *"…there is no convincing evidence that the addition of DHA to infant and follow-on formulae has benefits beyond infancy on any functional outcomes".* The proposal of the Panel to add DHA to infant formulae and follow-on formula is based on DHA's structural role in the nervous tissue and the retina and its involvement in normal brain and visual development. The need for the developing brain to accumulate large amounts of DHA in the first two years of life and the consideration that the intake of pre-formed DHA

generally results in an erythrocyte DHA status more closely resembling that of a breastfed infant than is achieved with ALA alone.

Maternal DHA and its effects on placental structure and functional development

In order to effectively exchange nutrients and waste products between mother and fetus throughout gestation, optimum placental growth and development are critical for a successful pregnancy [82]. Normal placental development and function are critical fetal development and its survival and for the mother's well-being. This is best exemplified by the fact that nearly all human pregnancy complications are linked to aberrant placental development with a deranged vasculature. Insufficient uterine blood supply is associated with a higher risk for preterm delivery, preeclampsia (PE), and intrauterine growth restriction (IUGR). Defective invasion of the uterine spiral arteries by extra villous trophoblasts is directly involved in preeclampsia, a major complication of human pregnancy[83]. Invasive extravillous trophoblasts of the human placenta are critically involved in remodelling of the uterine spiral arteries to increase blood flow to the feto-placental unit. The human trophoblastic invasion is temporally restricted to early pregnancy and spatially confined to the endometrium, the first third of the myometrium, and the associated spiral arterioles. Preeclampsia has been associated with aberrant angiogenesis and placental dysfunction resulting in adverse pregnancy outcomes. Angiogenesis involves the formation of new branches from pre-existing vessels is critical for placental development and vasculature [84]. The mechanisms underlying extravillous trophoblast invasion have not been fully established, but it is known that many molecular pathways are involved. Several factors are involved in this angiogenic process, including vascular endothelial growth factor (VEGF), angiopoietin-like protein 4 (ANGPTL4), platelet-derived growth factor (PDGF), platelet-activating factor (PAF), and also matrix metalloproteinases[85]. Latest studies demonstrated that DHA, apart from its important fetal brain and retinal development requirements during the last trimester of pregnancy, may also be involved in the early placentation process. DHA was shown to stimulate the tube formation *in*

vitro (as a measure of angiogenesis) in first-trimester human placental trophoblast cells, HTR8/SVneo cells [86,87]. DHA is the most potent stimulator of tube formation compared with EPA, LA, and oleic acid (OA) in HTR8/SVneo cells [86,88]. DHA-induced tube formation was mediated via increased VEGFA synthesis, whereas other fatty acids such as ARA, EPA, OA-stimulated tube formation via increased synthesis of ANGPTL4 in these cells. This is in contrast to the fact that n-3 LCPUFAs down-regulate the production of VEGFA and inhibit angiogenesis in other cells [89,90]. Both EPA and DHA have potent anti-angiogenic effects in cancer cells by inhibiting production of many angiogenic mediators such as VEGF, PDGF, COX-2, PGE_2, nitric oxide (NO)[89,91]. EPA increased VEGF-A synthesis in 3T3–L1 cells through both GRP120 and peroxisome proliferator–activated receptor γ (PPARγ) pathways [92]. In contrast, 4-hydroxy-docosahexaenoic acid (4-HDHA), a metabolite of DHA, was shown to inhibit endothelial cell angiogenesis via PPARγ pathway [93]. It is unlikely that the DHA metabolites are involved in the tube formation and/or VEGF synthesis in trophoblast cells as lipid metabolic genes (COX-2 and CAV-1) and fatty acid receptor genes (FABP4, GPR120, GPR40) were not upregulated by DHA[88]. Mechanisms responsible for EPA, DHA and ARA- induced increase in tube formation in these cells are not yet fully known. However, these LCPUFAs in trophoblasts increased COX-2 gene expression, which may be associated with an increase in angiogenesis[88]. DHA may also help the early placentation process by increasing angiogenesis. This is in contrast with the generally observed inhibitory effects of n-3 LCPUFAs on angiogenesis in many cell types, including tumors. DHA's mechanism responsible for the increased expression of VEGFA in placental trophoblast cells is not known at present. Expression of VEGFA by DHA however, is unique as its mRNA is induced by a variety of growth factors and cytokines, including PDGF, EGF, TNFα, TGF-β1, and IL-1β, but not by any fatty acids. DHA-induced VEGFA expression was not accompanied by COX-2 and HIF1 genes in these cells, indicating that DHA metabolites per se may not be involved in the VEGFA expression. Since the PPARγ ligand did not stimulate VEGFA expression in these

cells, it is unlikely that DHA stimulation of VEGFA expression involves PPARγ. The mechanisms that determine the angiogenic capacity of DHA compared with other fatty acids may underlie important differences in the mechanism of actions of these structurally different fatty acids. Based on available data, it can be postulated that DHA stimulates expression of FABP4 that has been demonstrated as a target protein for VEGF in other cell system. Further work is required to understand DHA's differential effects vs. other fatty acids in endothelial, tumour cells and placental first-trimester trophoblast cells.

During the last trimester of pregnancy, the supply of DHA is of great importance for fetal brain and retinal development[87,94]. Because of DHA's limited endogenous synthesis, the growing fetus mostly depends on the placental supply of maternal DHA [94]. Placental trophoblasts take up maternal plasma free fatty acids (FFAs) via several fatty acid transport proteins (FAT/CD36, FATPs, and FABPpm) and intracellular fatty acid-binding proteins (FABPs)[95]. The placental DHA uptake and transport mechanism are not fully understood yet; however, a preferential DHA transport system involving placental plasma membrane fatty acid-binding protein (p-FABPpm) was demonstrated [96,97]. p-FABPpm has a high affinity for LCPUFAs only[98]. Recent data suggest brain DHA uptake is facilitated by a transporter major facilitator superfamily domain-containing 2A (MFSD2a) located in the blood-brain barrier vessel that is specifically involved in transporting lysophosphatidylcholine form of DHA from the circulation into the brain. The role of this transporter in the placenta is not clear at this moment. Expression of the MFSD2a gene during pregnancy is being used as a biomarker in predicting fetal neurodevelopment [99]. Maternal MFSD2a levels in blood was positively associated with Z-score of head circumference at multiple time point at the first year of the neonate. Not much is known about the cellular localization of MFSD2a in human placenta yet, although its expression is altered in the placenta of GDM and PE. Whether the expression of the protein in normal pregnancy could be an indicator for DHA deficiency to the fetus brain still needs to be ascertained. Placental MFSD2a transporter expression was decreased and

correlated to decreased DHA in cord blood of women with gestational diabetes indicates its role in contributing to materno-fetal DHA transport [96,100,101].

Several observational studies confirmed a higher incidence of preeclampsia in patients with low n-3 fatty acids content of red blood cells [102]. Moreover, increased consumption of n-3 LCPUFAs during pregnancy was found beneficial for overall fetal development and the risk of early delivery and preeclampsia[2,103]. A positive association between placental DHA levels with placental weight was reported in preterm [104]. Maternal DHA content is, therefore not only important for fetal growth and development but may also play an important role in early placentation by stimulating first trimester trophoblasts mediated processes. At present, many international scientific societies recommend the use of pre-formed DHA for the prevention of premature birth[105], based on data from meta-analyses and large RCT studies [106,107]. However, most of the n-3 LCPUFAs trials were carried out from 16th- 20th week of gestation, more studies are required whether supplementation of DHA at the pre-conception stage or earlier than 14th week of gestation (at the earlier placentation stage) can ensure the optimum placentation and avoid preeclampsia related disorders.

During the third trimester, DHA accumulates in large amounts in developing fetal brain and retina[108,109], which may correlate with normal eyesight and brain function[108]. Very low birth weight (VLBW) babies remain DHA deficient (since they missed the DHA's placental supply during the last trimester) for an extended period after birth. Several studies demonstrated the benefit of n-3 fatty acids supplementation during pregnancy in terms of proper development of the brain and retina[110]. N-3 LCPUFAs supplementation during pregnancy also resulted in a slightly longer gestation period and somewhat higher birth weight[111,112]. The results on higher birth weight and length of gestation were confirmed in different meta-analyses on n-3 LCPUFAs supplementation during pregnancy[111]. The effect of DHA supplementation on gestation length in the general population of pregnant women had

mixed results[69,113]. Because of the studies' heterogeneity and the great variability in the observational studies, more studies are needed to confirm the findings on the supplementation of n-3 LCPUFAs.

Moreover, marine fish's low consumption was a decisive risk factor for preterm delivery [114]. In a multi-centre trial involving women with a preterm delivery history, consumption of n-3 LCPUFAs significantly lowered the rate of recurrent preterm delivery [111]. The effects of n-3 LCPUFAs supplementation in high-risk pregnancies are, however, controversial[113]. In another study, the daily intake of n-3 LCPUFAs or placebo did not prevent the recurrence of IUGR or pregnancy-induced hypertension [115]. The sample size is essential for determining the measurable differences in birth outcomes by supplementation of n-3 fatty acids during pregnancy[107,109]. It is important to consider that the higher birth weight reported for n-3 LCPUFAs supplementation during pregnancy was probably due to the greater length of these pregnancies [116]. The most important observation is, however, the amount of n-3 fatty acids in the fetus was correlated with the amount ingested by the mother, so it is essential that the mother have adequate intakes of n-3 fatty acids [117]. Optimizing LCPUFA provision in the postnatal period improved vision and brain development in VLBW infants [118].

Maternal intakes of DHA and its impact on fetal brain development

Despite the several studies that supported the nutritional and metabolic significance of n-3 PUFA, until the early 1980s there were some doubts about the real importance of these fatty acids, particularly about the essentiality of ALA, the precursor of n-3 LCPUFA. This doubt was dispelled after the first report on the deficiency of ALA recorded in 1982, related to the case of a 6 year-old girl who had undergone surgical resection of part of her small intestine, who received total parenteral nutrition (75.9% LA and 0.66% ALA). After five months of

receiving parenteral nutrition, the girl presented neurological disorders, particularly numbness in his extremities, paresthesia, and difficulty for walking, leg pain, and blurred vision. However, when the parenteral formula was replaced for a formula with a higher content of ALA (42.4% LA and 6.9% ALA), the neurological disorders were totally reversed[119]. Based on this case report, Holman and coworkers stated that ALA was an essential fatty acid and the minimum dose for preventing the symptoms caused by ALA deficiency was estimated to be in the range of 0.5–0.6% of total energy intake[119]. Subsequently, a study done with institutionalized elderly patients who received formula based on corn oil (61% LA and 0.5% ALA) through nasogastric feeding showed they did not develop the neurological alterations observed in the 6 year-old girl, but developed dermatological disorders, particularly dermatitis, and flaky skin, together with deficient levels of circulating EPA and DHA. However, when 0.3% ALA was added to the formula, the skin symptoms were resolved in four weeks, along with normalization in plasma levels of EPA and DHA[120]. Based on these results, researchers argued that for older adults the lowest daily intake of ALA should be 0.2–0.3% of the energy/day and for EPA plus DHA should be 0.1–0.2% of the energy/day, indicating that in the absence of EPA and DHA the endogenous biosynthesis of these fatty acids from ALA is significantly increased [121]. Based on these data and in relation to the importance of DHA in the nervous system, particularly the brain and retina, currently there is a relative agreement that humans are only able to transform 1% of ingested ALA into DHA, this conversion being more efficient and critical during the first years of life[122] . After the delivery, breast milk is the only food that provides all the essential nutrients for the newborn, with the contribution of necessary n-3 and n-6 LCPUFA to ensure optimal brain development, thus acquiring particular importance the maternal nutrition during pregnancy and lactation [123-125].

The relative content of DHA in human milk varies significantly in different populations, being values from 0.1% to up 1% of the total fatty acids present in human milk, variation primarily explained by the eating of fish or other seafood or from land animals that have been fed fish meal and/or fish oils [38,126]. It is noteworthy that in the past three decades, the levels of DHA in breast milk has been significantly reduced in the general western population primarily due to low consumption of DHA containing foods, among these are marine fatty fish like tuna, mackerel, codfish, salmon, sardine and anchovy, the most [127]. During pregnancy, the minimal recommended ingestion of DHA according to Food and Agriculture Organization (FAO) is 200 mg/day[128], but there is no universal consensus because it is difficult to obtain this ingestion due to the multiple reasons discussed in the next section. However, when pregnant women are advised or received dietary counseling about the physiological relevance of DHA, it is possible to observe a significant increase in the consumption of those foods and/or supplements that provide such important n-3 LCPUFA[129]. Dietary counseling about the benefits of fish consumption during pregnancy increases the consume n-3 LCPUFA in the diet[130]. FAO and many researchers have established that fish consumption rich in n-3 LCPUFA may override the possible negative effects of low metal or other organic contaminants eventually present in these foods[131]. Weekly consumption of two portions of fish rich in DHA, such as salmon, tuna, anchovy or mackerel, may contribute significantly to fulfill the minimal recommendation for the fatty acid during pregnancy, also increasing DHA levels of umbilical blood[132,133]. Low ingestion of foods that naturally provide DHA (such as fatty or blue fish) during pregnancy is critical to obtain enough fatty acid levels, which is reflected in low levels of DHA in umbilical blood[134]. *Trans* fatty acid ingestion may also decrease the availability of n-3 LCPUFA, such as DHA, to the mother and her child[135]. Policies to develop strategies to increase DHA consumption to the population, especially pregnant and nursing women, are high priority. The optimal ratio of n-6/n-3 PUFAs

is also important for cognition and brain development. Higher levels of n-3 PUFAs in colostrum are positively associated with infant mental development, as well as a higher ratio n-3/n-6 PUFAs[136]. Furthermore, LA levels in colostrum are negatively associated with child cognitive scores at ages 2 and 3 years, independently of breastfeeding duration[137].

The DHA uptake system in human brain

Usually non-esterified DHA is the major plasma pool supplying the brain with DHA[138,139]. In plasma, DHA is present in non-esterified form and esterified to lysophosphatydilcholine pools in similar amounts. The brain maintains levels of fatty acids via the uptake of plasma free fatty acids [140]. Astrocytes and endothelial cells, two major components of the blood brain barrier, are the major contributors to the transportation of fatty acids from the circulation to brain. The uptake of fatty acids into brain may occur by distinct or overlapping mechanisms, including passive diffusion a saturable transport process. FFAs after release from the proteins, carried through the plasma by albumin and circulating lipoproteins. At the inner surface of endothelial cell membranes, a small portion is delivered into the subcellular compartments for further metabolism, while most of the fatty acids diffuse into the cytosol with or without the aid of membrane proteins. **Figure-1** shows the putative fatty acid transport system of the brain. There are four classes of fatty acid transport proteins involved in transportation in adult brain, including fatty acid translocase (FAT/CD36), plasma membrane -fatty acid binding proteins (FABPpm), fatty acid transport proteins (FATPs) and cytoplasmic FABPs. Additionally, MFSD2a is newly identified as a DHA transporter in brain. Even though there is a preferential uptake by the brain of DHA esterified in lysophosphatydilcholine, this is not the major mechanism by which DHA is incorporated into the brain. Despite being an abundant fatty acid

Figure-1: DHA uptake system of the brain

DHA is carried through the plasma by albumin and circulating lipoproteins. There are four classes of lipid transport proteins are involved in DHA uptake of the brain that includes fatty acid translocase (FAT/CD36),

plasma membrane fatty acid-transport proteins (FABPpm), and fatty acid transport proteins (FATPs) and cytoplasmic FABPs. MFSD2a, a specific protein, can transport plasma DHA-lysophosphatidylcholines (LPCs) but not other form of DHA across the blood-brain barrier to the neuron.

Fig.1
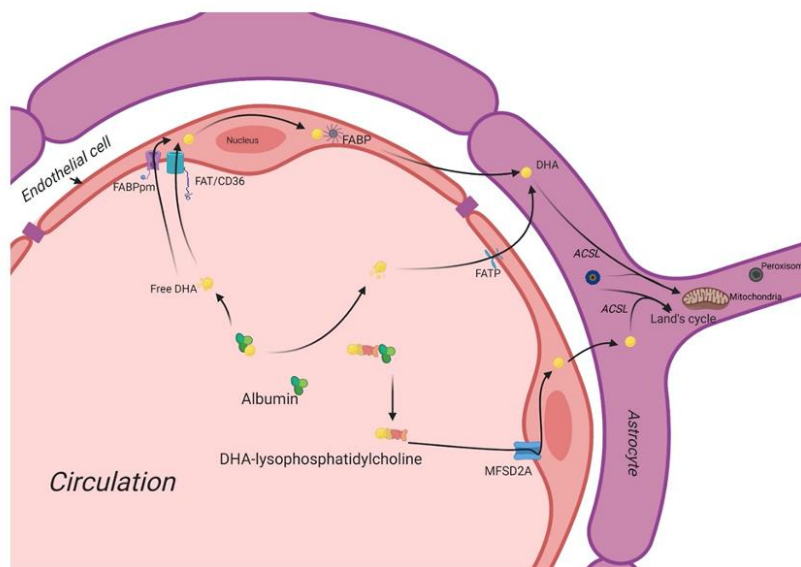
in brain phospholipids, DHA cannot be synthesized de novo in the brain and must be imported across the blood-brain barrier, but mechanisms are not well known yet. A member of the major facilitator superfamily MFSD2a (previously known as an orphan transporter) is identified as the major transporter for DHA uptake into the brain. MFSD2a is found to be expressed exclusively in the endothelium of the blood-brain barrier of micro-vessels. Lipidomic analysis indicates that MFSD2a-deficient (MFSD2a-knockout) mice markedly reduced DHA levels in the brain accompanied by neuronal cell loss in hippocampus and cerebellum, as well as cognitive deficits and severe anxiety, and microcephaly. Unexpectedly, cell-based studies indicate that MFSD2a transports DHA in the form of lysophosphatidylcholine (LPC), but not unesterified fatty acid, in a sodium-dependent manner. Notably, MFSD2a transports plasma pool LPCs carrying long-chain fatty acids such as LPC oleate and LPC palmitate, but not LPCs with less than a 14-carbon acyl chain. Long-chain acyl-CoA synthetases (ACSLs) are also involved in brain DHA uptake [96,97].

Brain development involves an incredible increase in the de novo synthesis and accretion of DHA, mediated by several transcription factors, including SREBP. The mechanism of DHA uptake in the brain has recently been proposed after localizing the MFSD2a transporter in the mouse brain and later observed in humans. Plasma-derived LPC-DHA uptake is driven by MFSD2a, leading to DHA enrichment in the phospholipid pool during pre and post-natal brain development. Once saturated, the phospholipid DHA pools weaken SREBP activity that leads to a decrease in lipogenesis. Thus, in normal physiology, the activity of MFSD2a is regulated by SREBP to maintain a balance between de novo lipogenesis and exogenous uptake of LPC-DHA [141].

DHA deficiency during fetal brain development and its impact on cognitive functions

Maternal intake of DHA during pregnancy and lactation ensures adequate maternal reserve deposited during pregnancy in particular to support six-month breast-fed post-natal life. Maternal adipose reserves during pregnancy covers the increasing demand for DHA in early post-natal stage. Although it is difficult to estimate the quantity of DHA, require in the diet for optimum brain development, the study from Kuiper et al. [142]first estimated the absolute requirement of DHA, ARA, and LA at 25 (conceptual age), 35 (preterm), and 45 (term) weeks. Based on the mother's data fed on the western diet, DHA accretion rate was found 42mg/day at the last five weeks of pregnancy. The accretion of DHA was found double in the last five weeks of pregnancy compared with first thirty-five weeks together. The DHA deficiency during brain development is largely determined by DHA accretion rate in the brain during term and preterm conditions. Thus, it is important to devise DHA requirement for preterm babies from the mother's data from a different ethnic background. The majorities of the available data that suggest DHA's optimal requirement during brain development in infants are fed on

Western diet. In a scenario with high LA intake in the diet will further enhance the need for pre-formed DHA in infant formula. In order to fulfill enough maternal reserve of DHA, intake may be required well before conception.

In developing populations, where pregnant women's usual diet is low in fats in which n-6 PUFAs are predominantly present with little intake of ALA or DHA as n-3PUFAs, most of the women start pregnancy with inadequate or poor n-3 PUFA status in their reserve. Under such a scenario, where a maternal reserve of DHA is low, endogenous synthesis of DHA from precursors is inadequate, can result in an insufficient supply of DHA to the fetus that may affect DHA accretion in the brain during the brain development phase of the neonate. DHA's placental deficiency can be correlated with the lower DHA accretion in the brain of the babies born preterm in developing population. There is no clinical data available about DHA accretion in the brain and neonatal's cognitive performance from the mother fed on n-3 deficient diet in such population. Therefore, optimal maternal intake of n-3 LCPUFAs is essential to fulfill neonatal requirement of DHA for the first six months. Maternal intake of DHA is correlated with problem-solving skills of the children in some aspects. It was argued that inadequate intake of DHA can affect rapid accretion of DHA in the human brain, particularly when brain growth is maximal as with the third trimester or first six months of life. Adequate DHA during this period ensures maturation of specific brain domain of prefrontal cortex that may support the problem-solving skills.

The impact of DHA deficiencies on cognitive functions are extensively studied using animal models in vivo. DHA's deficiency can influence the endogenous synthesis of DHA in the brain and its impact on synaptic plasticity. A recent study with DHA deficient mice by silencing ELOVL2, an enzyme that is responsible for endogenous synthesis of DHA from its

precursors, showed downregulation in the expression of neural plasticity factors (BDNF, Arc-1) with a concomitant increase in the pro-inflammatory markers (TNF-α, IL-1β, iNOS) in the cerebral cortex of the DHA deficient mice [143]. The altered expression of these factors is also associated with the learning and memory function in the brain. The endogenous DHA deficient mice showed an alteration in microglial architecture and cytokine factors without involving astrocytes indicates that resident immune cells are affected in the brain by endogenous DHA deficiency. The replenishment with DHA restored the physiological expression of neuroinflammatory and neuroplasticity factors in the cerebral cortex. These data indicate that DHA plays a critical role in neuroimmune communication in brain function and synaptic plasticity. The damaging effects of n-3 PUFA deficiency on brain lipid composition and memory performance were evidenced in LPS induced rats models that suggest *in utero* n-3 PUFAs deficiency could be a potential risk factor for the neurodevelopmental disorders [144]. The n-3 PUFA deficiency in rats shows downregulated glutamate receptors and upregulated pro-inflammatory TNFα gene expression in the central nervous tissue independent of their effects on membrane composition [145].Chronic deficiency of DHA over multiple generations during brain spurt affects the process of neurodevelopment by modulating the neuronal cell growth and differentiation as well as neuronal signaling. The deficiency may cause a functional deficit in the offspring's learning and cognitive efficiency by reducing intellectual potential and enhancing the risks of neurological diseases in adult life [146]. The omega-3 fatty acid deficiency disrupts the peripheral balance of pro- and anti-inflammatory state in the brain due to altered systemic AA: DHA ratio [147]. The excess ARA generates high prostaglandin concentrations, leukotriene, and thromboxane that lead to a pro-inflammatory state in the brain that disrupts the balance of anti (n-3) and pro-inflammatory (n-6) eicosanoids due to alteration in GPR receptors' signaling [148]. In the animal model, DHA's maternal deficiency revealed reduced telencephalon structure in the hippocampus region [149]. Such a deficiency state affects

region specific brain development areas where the cerebral frontal cortex region is affected mostly, leading to hyper motor activity, reduced learning ability and altered monoamine transmission [150]. The maternal DHA deficiency affects the offspring's brain development in a gender-specific manner due to differential efficiency of endogenous DHA converting enzyme in males and females. As the endogenous conversion of DHA from its precursor are more efficient in female newborn due to presence of estrogen, therefore, the infant male is more susceptible for the risk of brain disorders such as ADHD, Autism etc. in their later life [151]. The maternal DHA deficiency state profoundly affects behavioral change in feed intake, anxiety and stress response in the offspring [152]. The omega-3 deficiency state triggers sucrose-motivated food intake preference due to alteration in the brain-rewarding pathway that may prompt children to consume calorie-dense foods [153]. Chronic deficiency of omega-3 fatty acids for multiple generations induces anxiety-related stress behavior in the offspring due to altered expression of neuropeptide Y-1 receptor and glucocorticoid receptor in the pre-frontal and hippocampus of the rat brain [146,154]. Data from several in vivo studies suggest that DHA promotes neurogenesis by improving the membrane fluidity in the structural domain of the hippocampus, pre-frontal cortex and hypothalamus region to stabilize the neurodevelopment circuitry network required for learning and memory recognition processes [155]. Dietary deficiency of n-3 fatty acids during in utero development and the postnatal state has detrimental effects on cognitive abilities [69,156]. Animal studies strongly suggest that dietary deficiency of DHA increases the risk for neurocognitive disorders and that diets enriched with DHA increases learning and memory, and are protective against cognitive decline during aging. However, whether increased intake of DHA can decrease the risk of brain disorders requires further investigations.

Maternal DHA supplementation and brain development

During pregnancy, the importance of DHA for fetal brain development has been shown in

a large observational study ($N = 11,875$). The study found that children born to mothers with a higher intake of seafood during pregnancy showed an improved fine motor skills, greater pro-social behavior, higher verbal intelligence, and higher social development scores at eight years of age [61]. A recent longitudinal cohort concludes that higher DHA status during pregnancy and lactation is associated with infant's problem-solving skills at 12 months[157]. But Crozier didn't find any relationship between DHA level during pregnancy and cognitive performance of 4- or 6-years old children[157].

Randomised controlled trial (RCT) found that taking 200mg DHA orally daily for 4 months after delivery caused children's higher cognitive abilities at 5 years of age[158]. Ogundipe et al. showed that 300mg/day DHA supplementation on the last trimester of pregnancy is correlated with an infant's brain volumes (on MRI scan)[159]. DHA (600mg/day) supplementation from 14.5 weeks of pregnancy until the delivery on KUDOS study found improved visual attention in infancy but no consistent long-term benefit in childhood[160]. DHA (120mg/day) supplementation along with EPA (180mg/day) from 20th week of pregnancy until 30th day of postpartum period among 18-35 years old women in Iran found primary neurodevelopment improvement among 4 to 6 months old children [161]. However, another RCT from Australia did not find any effect among the toddlers at 18 months of age after intake of 800mg DHA in 2399 pregnant women during $<21^{st}$ weeks of gestation to delivery[14]. Maternal supplementation of DHA (400mg/day) during pregnancy positively reflected to the child (5-6yr) performance on language skill and short-term memory [162]. This study showed that the effects of maternal DHA intake during gestation observed in 18 months infants, were not identified at 5·75 years. DHA insufficiency's long-term potential effects may be too small to detect, or it is possible that the DHA intake was not low enough during gestation to have lasting effects. Human brain development begins as early as the third week of gestation. A considerable DHA accretion has been reported to occur during the brain growth spurt beginning in the third trimester as it is unknown whether a low DHA supply early during

gestation compromises embryonic brain development or not. Most intervention studies of prenatal DHA and infant neurodevelopment began supplementation in the second trimester, and thus intervention at 16 weeks of gestation may have been too late if DHA is important for early structural brain development. However, the inverse association of child Beery scores with maternal erythrocytes 22: 4n-6 and 22: 5n-6 suggest that visual–motor integration development is sensitive to low prenatal DHA, consistent with the time course of brain maturation, where maturation occurs in the visual cortex before the prefrontal cortex. Pregnant women who consumed DHA-rich eggs (135 mg DHA/egg), compared to pregnant women consumed non enriched-eggs (18mg DHA/egg), showed higher erythrocyte and umbilical cord DHA levels [163]. Pregnant women who consumed a cereal-bar enriched with DHA (300 mg DHA/bar) delivered babies with increased visual acuity until four months postpartum[164], and showed better capacity to resolve problems and an improved organization of their dream[165]. Recommendations to increase fish consumption are counteracted by the fear of metal contamination of these foods. Indeed, many health professionals recommend avoiding or reducing fish consumption, especially to pregnant women. Despite some organoleptic problems, because the reconstituted product developed some uncomfortable smell that it is not accepted for some mothers, the formula has proved to increase DHA content of breast milk[166]. A new version of the formula overcomed this organoleptic limitation and transformed it an inexpensive way to increase DHA availability for the Chilean pregnant and nursing population[167]. Available data based on randomized controlled trials suggest beneficial effects of maternal supplementation of DHA on neonatal growth and cognitive development (**Table-1**).

Several RCTs found that DHA supplementation on term and pre-term infants found a significant outcome on visual development during infancy[168]. **Table-2** presents the

postnatal supplementation of DHA on the neonatal visual, verbal and cognitive development:

a collection of Randomized Controlled Trials. A meta-analysis of RCTs on routinely

Table 1 Maternal supplementation of DHA on the neonatal growth and cognitive development: a consolidated Randomized Controlled Trials

Subject, sample size, location	Dosages, Duration	Primary outcome	References
Pregnant women, n=350, USA	DHA 600mg per day, <20wk to delivery	Gestational duration ⬆ Birth size ⬆	Carlson et.al. 2013[40]
Pregnant women, n=315, Germany and others	DHA 500mg and EPA 150mg per day, <20wk to delivery	Visual coordination 2.5yr. children ⬆ Cognitive development 5.5yr. children⬆	Dunstan et.al.2008 Escolano et.al.2011[47]
Pregnant women, n=300, UK	DHA 300mg, EPA 42mg, ARA 8.4mg per day for 12wks from third trimester	MRI of infant (n=86) at birth show a correlation with DHA and brain volume ⬆	Ogundipe etal.2018[159]
Pregnant women, n=271, Canada	DHA 400mg per day, 16wk to delivery	Maternal DHA correlates on language and short-term memory development of 5.79yr children ⬆	Mulder etal.2018[162]
Pregnant women, n=1094, Mexico	DHA 400mg per day, 18-22wk to delivery	Birth size and head circumference at birth ⬆ Attention of 5yr pre-school children⬆	Ramkrishnan et.al. 2010[109]
Pregnant women, n=2399, Australia	DHA 800mg per day, <21wk to delivery	No effects on cognitive and language development in 1.2yr infant	Makrides etal.2010[14]
Pregnant women, n=301, USA	DHA 600mg per day, 14.5wk to delivery	Cognitive behavior 10mo to 6yr ; Visual attention ⬆ No long-term beneficial effects	Colombo et.al.2019[160]
Pregnant women, n=143, Norway	DHA 1183mg and EPA 803mg per 10ml per day, 18wk to post-delivery 3mo	Mental processing score at 4 and 7yr age ⬆ No effects on BMI at 7yr age	Helland et.al 2003[60]
Pregnant women, n=150, Iran	DHA 120mg and EPA 180mg per day, 20wk to post-delivery 1mo.	Primary neurodevelopment outcome of 4-6mo Infant ⬆	Ostadrahim etl al 2018[161]
Pregnant women, n=98, Australia	DHA 2200mg and EPA 1100mg per day, 20wk to delivery	Visual and coordination 2.5yr children ⬆	Dunstan etal.2008[47]
Pregnant women, n=30, USA	DHA 214mg as functional food, 24wk to delivery	Visual acuity 4mo infant ⬆	Judge et.al. 2007[164]

Abbreviations: DHA: Docosahexaenoic acid; ARA: Arachidonic acid; EPA: Eicosapentaenoic acid; Mo: Month; Yr: Year; Wk : Week; MRI: Magnetic resonance imaging

supplemented infant formula milk with DHA has found no beneficial role in neurodevelopmental outcomes [169]. A large trial (DHANI) carried out in India, where prenatal and 6 months of post-partum 400mg/day maternal DHA supplementation effects are measuring to evaluate the neurodevelopment outcome[170]. These data are now published in this special issue[171]. The study reported that the mean development quotient (DQ) scores in the DHA and placebo groups were not statistically significant after 12 months supplementation of mothers through pregnancy and lactation with 400 mg/d DHA[171].

Conclusions

The effects of DHA on the brain and cognitive development have been extensively investigated. The DHA, by virtue of its ability to control membrane fluidity, also modulates neuronal density, neurotransmitter concentration, and synaptic activity by regulating the brain's neuroinflammatory state. Increasing evidence suggests that DHA, the foremost important n-3 long chain fatty acid in the brain, has neurotrophic and neuroprotective properties. N-3 PUFA deficient diet during early development lowered the brain n-3 PUFA concentration. Together, this body of evidence supports the proposition that DHA deficiency in utero increases the vulnerability of brain development. Most evidence indicates that the DHA accumulation is mainly influenced by dietary intake, specifically of preformed DHA. Decreased intake of n-3 PUFA may lead to DHA deficiency states that could affect the offspring's metabolic phenotypes by altering placental phenotype, fetal adiposity, body fat distribution, energy utilization, musculoskeletal growth, brain-adipose signaling, lipid homeostasis, epigenetic stability, and systemic inflammation [172]. **Figure-2** shows Maternal source and delivery of DHA at different stages of development affects fetoplacental and fetal brain development.

DHA accumulates rapidly in the retina and cerebral cortex during the period of significant brain growth between the last trimester and the second year of life. Intervention studies have shown that improving maternal DHA nutrition decreases the risk of poor visual and neural development in infants and children. Several pieces of

Table 2 Postnatal supplementation of DHA on the neonatal visual, verbal and cognitive development: a collection of Randomized Controlled Trials

Subject, sample size, location	Dosages, Duration	Measured outcome	References
Term formula fed infant, n=343,USA	DHA (0.32%-0.96%), ARA (0.64%) from 1-9 day to 1yr	DHA (0.32%) group visual acuity ⬆	Birch et.al.2010[68]
Term infant, n=420, Australia	DHA 250mg and EPA 60mg per day, birth to 6mo	Accretion of DHA ⬆ Early development of language and communication skills ⬆	Meldrum et.al.2012[71]
Infant BW <1.5kg, n=141, Norway	DHA 32mg, ARA 31mg per 100ml human milk per day, 1 to 9wk after birth	Memory recognition and problem solving skills of 6mo infant ⬆	Henriksen etal.2008[69]
Pre-term infant, n=107, UK	DHA (0.5%) in supplemented formula from birth to 9mo	Verbal and intellectual coefficient of 9yr girl ⬆	Isaacs et al 2011[72]
Pre-school healthy children, n=175, USA	DHA 400mg per day for 4mo of 4yr old children	Higher blood DHA correlates comprehension, punctuation and vocabulary abilities ⬆	Ryan et al 2008[79]
Indigenous school children 3 to13yr, n=409, Australia	DHA 750mg, GLA 60mg per day for 20 school week	Scholar performance in 7-12yr children ⬆	Parletta et.al 2013[80]
Term infant, n=227, USA	Algal DHA 200mg per day for 4mo after delivery	Cognitive abilities in 5yr children ⬆	Jensen et. al 2010[158]
Pre-term infant, n=361, USA	Algal DHA oil (17mg/100kcal), fungal ARA oil (34mg/100ml) from birth to 4mo	Growth and development of pre-term infant till 118wk ⬆	Clandinin et.al 2005[173]
Pre-term 23-24wk infant, n=90, USA	Algal DHA 50mg per day from 1 to 6-7wk at discharge	DHA levels of pre-term infant comparable to term placebo ⬆	Baack et.al 2016[174]
ADHD 7-12yr children, n=90, Australia	DHA 1032mg or 108mg, EPA 264mg or 1109mg for 4mo	Reading and spelling correlated DHA levels in the blood ⬆	Milte et.al 2012[81]
Larger pre-term 30-37wk, BW	DHA(0.05%), ARA(0.1%) infant	Mental development index at 6-12mo ⬆	Fang.et.al 2005[70]

>2kg, n=27, Taiwan	formula for 6mo after birth		
Breastfed 6mo infant, n=25-26, USA	DHA 115mg/100g baby food from 6mo to 12mo of breast fed infant	Maturation of retina and visual cortex at 12mo ↑	Hoffmen et.al.204[64]

Abbreviations: DHA : Docosahexaenoic acid ; ARA : Arachidonic acid ; EPA :Eicosapentaenoic acid ; GLA : Gamma linolenic acid; Mo : Month; Yr: Year; Wk : Week ADHD : Attention deficit hyperactivity disorder; BW: Birth weight

evidence support the notion that maternal transfer of DHA to the infant before and after birth, with short and long-term modulates neural functions. However, genetic variation responsible for endogenous conversion of DHA by fatty acid desaturases also influences essential fatty acid metabolism and may influence individuals' optimal requirements. Consideration of adequate DHA intake to include brain development, balanced intake of n-3 and n-6 PUFAs in gestation and lactation, and optimal fatty acid nutrition during pregnancy is required for infant neurodevelopment. Premature infants have a deficit in DHA shortly after delivery for several reasons including missing the third trimester DHA accretion. More studies are required to assess the optimal dosage of DHA, method of delivery and duration of supplementation to evaluate DHA intake in premature infants.

Figure-2: Maternal source and delivery of DHA at different stages of development affects fetoplacental and fetal brain development

DHA plays different roles in the gestation specific development of the feto-placental unit. During the first trimester, DHA possibly involves in decidual remodelling to establish early placentation by promoting the expression and secretion of angiogenic growth factors such as VEGFA, FABP4, ANGPTL4, leptin etc. DHA and its metabolites improve maternal-fetal immunocompetence by maintaining the oxidative stress, production of reactive oxygen species (ROS) and pro-anti-inflammatory balance in the maternal-fetal interface. DHA is delivered to the fetal brain during the third trimester via placenta and subsequently via breast milk. In addition to DHA's presence in the structural skeleton of the neonatal brain matter, mounting evidence suggests DHA helps

to several processes of brain development, including neurogenesis, synaptogenesis, brain plasticity, inflammatory signalling, neuroprotection etc.

Fig.2
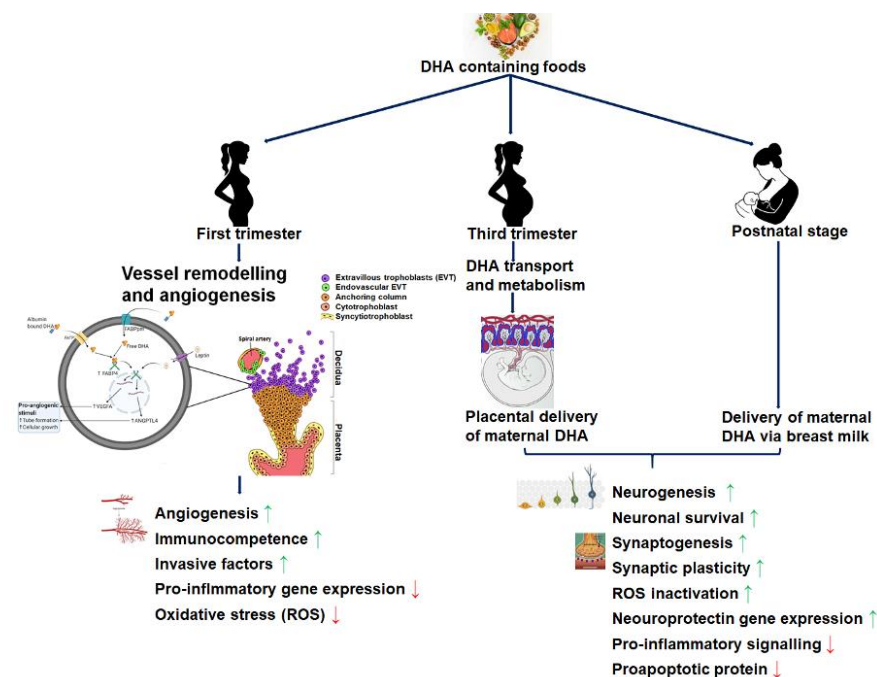
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