Among the various fatty acids, the Polyunsaturated Fatty Acids (PUFA) have immense physiological benefits and reduce the risk of many chronic disease. They are widely used as supplements to mitigate the significant morbidity and mortality caused by degenerative diseases of the cardiovascular system and brain. Out of several PUFAs, only two fatty acids are known to be essential for humans: ALA (α-Lenolenic acid, ω-3 or n-3 FA) and LA (Lenoleic acid, ω-6 or n-6 FA) which cannot be synthesized in human due to lack of the Δ12 and Δ15 fatty acid desaturases, responsible for converting oleic acid (18:1n-9) into LA (18:2n-6) and ALA (18:3n-3) (Tinoco, 1982; Holman, 1986). These are short chain polyunsaturated fatty acids (SC-PUFA) obtained from diet and can be converted to long chain polyunsaturated fatty acids (LC-PUFA) by subsequent desaturation and elongation in humans. However, in vivo studies in humans show that conversion efficiency of ALA to eicosapentaenoic acid (EPA; 20:5n-3) and subsequently to docosahexaenoic acid (DHA; 22:6n-3) is low asymptotically equal to 5% to EPA and <0.5% to DHA (Igarashi et al., 2006; Williams and Burdge, 2006; Kapoor and Patil, 2011; Brenna, 2002) in men, but greater than this in women (Burdge, 2004).

1. Biosynthesis
Liver is the major organ for conversion of the two C18 PUFAs, LA and ALA, to arachidonic acid (AA; 20:4n-6) and DHA respectively. ALA is converted to stearidonic acid (18:4n-3) by FADS2 (Fatty acid desaturase 2) or Δ-6 desaturase, which is then elongated to eicosatetraenoic acid (20:4n-3). Further desaturation by FADS1(Fatty acid desaturase 1) or Δ-5 desaturase yields EPA. The first three enzymatic steps that form ARA and EPA from LA and ALA respectively are thought to be carried out by the same three enzymes and thus substrate like LA and ALA compete with each other for synthesis of their respective LC-PUFA products. Again dietary ALA inhibits Δ-6 desaturation of LA and also the desaturation products AA, EPA, and DHA inhibit Δ-6 desaturation of LA and Δ-5 desaturation of DGLA (dihomo-gamma-linolenic acid) (Bezard et al., 1994). Δ-6 desaturase is the rate limiting enzyme in this pathway and the activities of Δ-6 and Δ-5 desaturases are regulated by nutritional status, hormones and feedback inhibition by the end products, creating a complex control network for endogenous long-chain PUFA synthesis. High LA-diet inhibits synthesis of n-3 PUFA and high ALA diet inhibits further conversion of ALA to DHA (Gibson et al., 2011). EPA can be elongated further by two elongation steps to 24:5n-3 via 22:5n-3 (docosapentaenoic acid – DPA). 24:5n-3 is desaturated by Δ6-desaturase forming 24:6n-3. All these reactions occur in the endoplasmic reticulum of cells. 24:6n-3 is then transferred from the ER to the peroxisome where it is β-oxidized to form DHA (Voss et al., 1991;
Brenna et al., 2010; Sprecher 2000; Burge and Calder 2005). DHA is then transferred back to the ER where it can undergo esterification, lipoprotein packaging and secretion to the blood. The detailed biosynthetic pathway is shown in Fig 1. Low rates of DHA synthesis from ALA appear to be a general characteristic of human metabolism, with the slowest step in (n-3) fatty acid de-saturation being at the conversion of EPA to DHA (Williams and Burdge, 2006). Interestingly, microalgae seem to be more efficient at synthesizing DHA by bypassing the last three steps and simply converting DHA from DPA using Δ-4 desaturase, in a single step (Monroig, 2013).

![Biosynthetic pathways of LC-PUFA. LC-PUFAs are derived from C18-PUFAs (LA and ALA, obtained from the mammalian diet) by alternate desaturation (red/orange enzymes) and elongation (blue enzymes) steps. These enzymes utilize both n-6 and n-3 substrates. n-3 LC-PUFAs undergo further metabolism through a β-oxidation step (green box) to the generate DHA. [from Nutrients, 2014, 6, 1993-2022.]

2. n-3 PUFA and human evolution

A possible link of DHA with the large brain size in human, stems from the series of evolutionary studies carried out by Trinkaus and coworkers. In early modern humans, a number of individuals showed evidence for the consumption of aquatic (marine and
freshwater) resources, which contains mainly DHA and other PUFAs. That nutrition plays an important role in human evolution, is reflected from a number of studies. Marine and estuarine ecosystem had mainly provided the proper stimulus to hominid brain to develop into a relatively large and neurologically complex organ (Cunnane et al., 1993). Plentiful, easily harvested seafood rich in DHA, available in land-water interface, got included in the diet of early modern humans (Crawford 1999, 2002) coinciding with the rapid expansion of cerebral cortex and grey matter thus increasing intelligence, development of language and tool making ability (Broadhurst et al., 1998). Modern Homo spp. are believed to be originated in Africa and specially associated with lake shore (lacustrine) environments in the East African Rift Valley (Broadhurst et al., 1998).

3. PUFA in health

Based on several randomized controlled trials and population cohort studies on cardiovascular disease, the American Heart Association, in 2009, recommended human diets should include high levels of both n-3 and n-6 PUFAs. n-6 PUFAs are modified to make the classic eicosanoids (Prostaglandins - PGD$_2$, PGE$_2$, PGF$_2$, PGI$_2$, Thromboxanes - TXA$_2$, TXB$_2$, Leukotrienes - LTA$_4$, LTB$_4$, LTC$_4$, LTD$_4$, LTE$_4$) while the n-3 FAs produce eicosanoids (Prostaglandins - PGD$_3$, PGE$_3$, PGF$_3$, PGI$_3$, Thromboxanes - TXA$_3$, TXB$_3$, Leukotrienes- LTA$_5$, LTB$_5$, LTC$_5$, LTD$_5$, LTE$_5$). Two major families of oxidation enzymes convert the 20C fatty acids into their respective eicosanoids: (i) cyclooxygenases (COXs) to the prostaglandins and thromboxanes, and (ii) lipoxygenases (LOXs) to leukotrienes and lipoxins. It is evident that the eicosanoids produced by n-3 PUFAs differ from those produced by n-6 PUFAs. n-6 derived eicosanoids promote inflammation, which is beneficial for controlling blood flow and vessel dilation, whereas n-3 derived eicosanoids exerts anti-inflammatory responses over reactive inflammatory response thus resolving the situation leading to the pathogenesis of inflammatory diseases. Both n-3 and n-6 FAs also produce the endocannabinoids which can affect mood, behavior and inflammation. n-6 can produce the non-classical eicosanoid, lipoxins, which have a number of immunomodulatory and anti-inflammatory actions. n-3 FAs, on the other hand, produce resolvins and protectin which down regulates inflammation and also Neuroprotectin D1, which can resolve oxidative stress and inflammation. Thus maintaining a healthy ratio of n-6 to n-3 fatty acids in the human diet is important and may influence blood clotting, smooth muscle contraction, inflammation and immune responses (Simopoulos, 1991; Simopoulos, 2008). Based on the limited studies in animals, children, and adults, a reasonable n-6 to n-3 ratio of 5:1 to 10:1 has been recommended (Aggett et al., 1991). According to WHO, both EPA plus DHA should be
consumed and either EPA or DHA alone is not recommended. For adult males and females 0.250 g/day of EPA plus DHA is recommended, whereas adult pregnant and lactating females need more minimum intake for optimal adult health and foetal and infant development which is 0.3 g/d EPA plus DHA, of which at least 0.2 g/d should be DHA (FAO, 2008). Although the concept of pharmaco-nutrition assess both n-3 and n-6 fatty acids as beneficial for human health, however, assessment of the effects of various PUFA containing dietary compounds in specific groups of patients or in defined clinical conditions, suggests n-3 FAs, particularly DHA to be the most important, having an integral role in the human health. They form lipid rafts affecting cellular signaling (Stillwell et al., 2005). They act on DNA, activating or inhibiting transcription factors such as NF-κB, which is linked to pro-inflammatory cytokine production (Calder et al., 2004).

4. What is Docosahexanoic acid?

DHA is a carboxylic acid (-oic acid) with a 22-carbon chain (docosa- is Greek for 22) and six (hexa) cis double bonds (-en-); with the first double bond located at the third carbon from the omega end. Its trivial name is cervonic acid while its systematic name is all-cis-docosa-4,7,10,13,16,19-hexa-enoic acid. The chemical structure of DHA is as follows:

![Chemical structure of DHA](image)

Studies have revealed that DHA is the primary structural component of human brain, cerebral cortex, skin, sperm, testicles and retina.

5. Source of DHA

Cold-water oceanic fish oils are rich in DHA which actually originated from the photosynthetic and heterotrophic microalgae consumed by these fish. This becomes increasingly concentrated in organisms by their feeding habits and further they came up the food chain. DHA is also commercially manufactured from microalgae: *Crypthecodinium cohnii* and another of the genus *Schizochytrium*. However, in strict herbivores and carnivores that do not eat seafood, DHA is manufactured internally from α-linolenic acid, manufactured by plants. Human gets DHA from diet like fish, fish oils, egg/dairy products and ALA from various plant products. Breast milk is the source of DHA for babies. α-linolenic acid content in various foods and oils (Table 1) and in fish and sea foods (Table 2) are shown below.
Table 1: Alpha-Linolenic Acid Content of Various Foods and Oils

<table>
<thead>
<tr>
<th>Source</th>
<th>ALA (g)</th>
<th>Source</th>
<th>ALA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nuts and Seeds</strong></td>
<td></td>
<td><strong>Legumes</strong></td>
<td></td>
</tr>
<tr>
<td>Almonds</td>
<td>0.4</td>
<td>Beans, common (dry)</td>
<td>0.6</td>
</tr>
<tr>
<td>Beechnuts (dried)</td>
<td>1.7</td>
<td>Chickpeas (dry)</td>
<td>0.1</td>
</tr>
<tr>
<td>Butternuts (dried)</td>
<td>8.7</td>
<td>Cowpeas (dry)</td>
<td>0.3</td>
</tr>
<tr>
<td>Chia seeds (dried)</td>
<td>3.9</td>
<td>Lentils (dry)</td>
<td>0.1</td>
</tr>
<tr>
<td>Flaxseed</td>
<td>22.8</td>
<td>Lima beans (dry)</td>
<td>0.2</td>
</tr>
<tr>
<td>Hickory nuts (dried)</td>
<td>1.0</td>
<td>Peas, garden (dry)</td>
<td>0.2</td>
</tr>
<tr>
<td>Mixed nuts</td>
<td>0.2</td>
<td>Soybeans (dry)</td>
<td>1.6</td>
</tr>
<tr>
<td>Peanuts</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pecans</td>
<td>0.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean kernels</td>
<td>1.5</td>
<td>Beans, pinto, sprouted (cooked)</td>
<td>0.3</td>
</tr>
<tr>
<td>Walnuts, black</td>
<td>3.3</td>
<td>Broccoli (raw)</td>
<td>0.1</td>
</tr>
<tr>
<td>Walnuts, English and Persian</td>
<td>6.8</td>
<td>Cauliflower (raw)</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Grains</strong></td>
<td></td>
<td>Kale (raw)</td>
<td>0.2</td>
</tr>
<tr>
<td>Barley, bran</td>
<td>0.3</td>
<td>Leeks (freeze-dried)</td>
<td>0.7</td>
</tr>
<tr>
<td>Corn, germ</td>
<td>0.3</td>
<td>Wheat, germ</td>
<td>0.7</td>
</tr>
<tr>
<td>Oats, germ</td>
<td>1.4</td>
<td>Lettuce, red leaf</td>
<td>0.1</td>
</tr>
<tr>
<td>Rice, bran</td>
<td>0.2</td>
<td>Mustard</td>
<td>0.1</td>
</tr>
<tr>
<td>Wheat, bran</td>
<td>0.2</td>
<td>Purslane</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Fruit</strong></td>
<td></td>
<td><strong>Seaweed</strong></td>
<td></td>
</tr>
<tr>
<td>Avocados, California (raw)</td>
<td>0.1</td>
<td>Spirulina (dried)</td>
<td>0.8</td>
</tr>
<tr>
<td>Raspberries (raw)</td>
<td>0.1</td>
<td>Soybeans, green (raw)</td>
<td>3.2</td>
</tr>
<tr>
<td>Strawberries (raw)</td>
<td>0.1</td>
<td>Soybeans, mature seeds, sprouted (cooked)</td>
<td>2.1</td>
</tr>
</tbody>
</table>

Data from Kris-Etherton et al. (2000)

Table 2: Fish and Seafood Sources of DHA plus EPA

<table>
<thead>
<tr>
<th>Source</th>
<th>DHA + EPA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td></td>
</tr>
<tr>
<td>Anchovy, European, raw</td>
<td>1.449</td>
</tr>
<tr>
<td>Carp, cooked, dry heat</td>
<td>0.451</td>
</tr>
<tr>
<td>Catfish, channel, farmed, cooked, dry heat</td>
<td>0.177</td>
</tr>
<tr>
<td>Cod, Atlantic, cooked, dry heat</td>
<td>0.158</td>
</tr>
<tr>
<td>Eel, mixed species, cooked, dry heat</td>
<td>0.189</td>
</tr>
<tr>
<td>Flatfish (flounder and sole), cooked, dry heat</td>
<td>0.501</td>
</tr>
<tr>
<td>Haddock, cooked, dry heat</td>
<td>0.238</td>
</tr>
<tr>
<td>Halibut, Atlantic and Pacific, cooked, dry heat</td>
<td>0.465</td>
</tr>
<tr>
<td>Herring, Atlantic, cooked, dry heat</td>
<td>2.014</td>
</tr>
<tr>
<td>Mackerel, Pacific and jack, mixed species, cooked, dry heat</td>
<td>1.848</td>
</tr>
<tr>
<td>Mullet, striped, cooked, dry heat</td>
<td>0.328</td>
</tr>
<tr>
<td>Perch, mixed species, cooked, dry heat</td>
<td>0.324</td>
</tr>
<tr>
<td>Pike, northern, cooked, dry heat</td>
<td>0.137</td>
</tr>
<tr>
<td>Pollock, Atlantic, cooked, dry heat</td>
<td>0.542</td>
</tr>
<tr>
<td>Salmon, Atlantic, farmed, cooked, dry heat</td>
<td>2.147</td>
</tr>
<tr>
<td>Sardine, Atlantic, canned in oil, drained solids with bone</td>
<td>0.982</td>
</tr>
<tr>
<td>Sea bass, mixed species, cooked, dry heat</td>
<td>0.762</td>
</tr>
<tr>
<td>Shark, mixed species, raw</td>
<td>0.843</td>
</tr>
<tr>
<td>Snapper, mixed species, cooked, dry heat</td>
<td>0.321</td>
</tr>
<tr>
<td>Swordfish, cooked, dry heat</td>
<td>0.819</td>
</tr>
<tr>
<td>Trout, mixed species, cooked, dry heat</td>
<td>0.936</td>
</tr>
<tr>
<td>Tuna, skipjack, fresh, cooked, dry heat</td>
<td>0.328</td>
</tr>
<tr>
<td>Whiting, mixed species, cooked, dry heat</td>
<td>0.518</td>
</tr>
</tbody>
</table>

Data from Kris-Etherton et al. (2000)
6. DHA composition in brain
The brain is the fattiest organ in the body as nearly 60% of dry weight of brain is contributed by lipid. Interestingly, 35-40% of this lipid in brain is PUFAs, mainly the long-chain PUFAs EPA, DHA and AA (Steenweg-de Graaff, 2015; Singh, 2005). DHA constitutes about 15% of the fatty acids in the human frontal cortex (Carver, 2001) suggesting an inevitable role of this ω-3 fatty acid in brain. Additional evidences of increase in brain weight during the initial postnatal months have been attributed to the high levels of PUFA in cerebellum, occipital and frontal lobes of brain (Clandinin et al., 1980a) and suggest that early developmental period are more sensitive to DHA. Accumulation of DHA in central nervous system occurs during the developmental period depending on the availability of DHA from circulating plasma. However, neurons are the main site for DHA accumulation which is readily derived from the release from astrocytic membranes under both basal and stimulated conditions (Garcia and Kim, 1997; Kim et al., 1999). Studies on C6-glioma cells suggest 5-HT2A receptors to be involved in the release of DHA (Garcia and Kim, 1997). On the other hand, it is quite difficult to deplete DHA from the neuronal membrane in adult mammals (Kim et al., 1999) even by Ca++ independent phospholipase A2 (iPLA2) activity which cause release of DHA from astrocytes (Strokin et al., 2003).

DHA is the major structural component of the neural plasma membranes being incorporated in the glycerophospholipids, at the sn-2 position, as the longest and most unsaturated fatty acid. At the subcellular level, the highest concentration of DHA is found in synaptic membranes followed by mitochondria and microsomes (Scott and Bazan, 1989). DHA is mainly found in ethanolamine plasmalogen (PlsEtn) and phosphatidylserine (PtdSer), whereas the majority of AA is incorporated into phosphatidylcholine (PtdCho). From PlsEtn and PtdSer, DHA is released by the action of PlsEtn-PLA2 and PtdSer hydrolyzing PLA2, respectively (Farooqui, 2009; 2010). The estimated daily basal turnover rate is 2-8% for DHA in brain of adult rat where esterified DHA is replaced with unesterfied DHA from plasma (Rapoport et al., 2001).

7. DHA transport in brain
Liver is a key “processor” and “distributor” of omega-3 FAs to the CNS (Bazan et al., 1985; Scott et al., 1989) corresponding to more than 30-fold higher rates of DHA synthesis in this organ (Leonard et al., 2002). The hepatic conversion of dietary ALA to DHA in healthy individuals is sufficient to meet the demands of DHA in the brain and retina (Rapoport et al., 2010; Scott et al., 1989). DHA derived from the diet or from hepatic elongation and
desaturation (Fig.1) is subsequently released into the bloodstream for delivery to the choriocapillaris behind the retina and the neurovascular units within the brain, as well as to other tissues (Fig. 2).

Fig. 2. Delivery of DHA in tissues. Systemic ALA is taken up by hepatocytes and transferred to the endoplasmic reticulum, where a series of desaturation and elongation steps occurs, leading to the formation of a 24-carbon, 6-double-bond FA [24:6, tetracosahexaenoic acid (THA)]. 24:6 is then conveyed to peroxisomes, converted to 22:6 by β-degradation, and delivered back to the endoplasmic reticulum. 22:6 (DHA) is then attached to the n-2 position of phosphatidyl choline to form a 22:6 phospholipid (22:6-PL), followed by release to the circulatory system for delivery to the choriocapillaris behind the retina and neovascular unit within the brain, as well as to other tissues. (Annu Rev Nutr. 2011, 31, 321–351.)

Like other FAs, non-esterified form of DHA is mainly transported to the brain (Purdon et al., 1997). It was initially thought that this free form of DHA, bound to albumin in the blood, very likely crosses the blood–brain barrier (BBB) (Spector, 1986). However recent evidences suggest that DHA crosses BBB via passive diffusion (Ouellet et al., 2009; Hamilton and Brunaldi, 2007). In liver, DHA associate either to albumin or to lipoproteins and is transported in the blood in bound form. DHA transport is facilitated by a number of membrane-associated and cytoplasmic proteins, including membrane proteins fatty acid translocase (FAT/CD36), plasma membrane fatty acid binding protein (FABPpm) and fatty acid transport protein (FATP). In the brain tissue, DHA cross the luminal and trans-luminal leaflets of the endothelial cells and the plasma membrane of neural cells by reversible flip-flop (Stremmel et al., 2001; Hamilton and Brunaldi, 2007) and is released in the brain through the action of endothelial cell lipoprotein lipase (Vilaro et al., 1990). In an in vitro model of BBB, which is reconstituted by coculturing of brain-capillary endothelial cells and astrocytes, it is also reported that lysophosphatidylcholine (lyso-PtdCho)-containing DHA can cross the BBB more efficiently than free DHA or DHA in triacylglycerol (Lagarde et al., 2001). DHA is mainly taken up by the endothelial cells from the circulation and transferred to astrocytes within the central nervous system; from there, it is incorporated into astrocytes or transferred to neurons (Fig. 3).
Most investigations on the supply and turnover of DHA in the brain have been mainly carried out in adult animals. Using positron emission tomography and intravenous \([1^{11}C]DHA\), it has been shown that the DHA uptake rate is between 2.4 and 3.8 mg/day in the adult human brain which was equivalent to the net rate of DHA consumption by the brain (Umhau et al., 2009). Inside the brain, DHA is first esterified and incorporated in membrane phospholipid particularly phosphatidylethanolamine (PE) and phosphatidylserine (PS). The estimated daily basal turnover rate is 2-8% for DHA in brain of adult rat where esterified DHA is replaced with unesterfied DHA from plasma (Rapoport et al., 2001).

*In vitro* culture studies suggest that unlike neurons, astrocytes are capable of synthesizing DHA (Bernoud et al., 1998; Moore et al., 1991; Moore et al., 1994) which is again influenced by the negative feedback loop for the availability of DHA, although some basic level of synthesis is continuous under all circumstances (Williard et al., 2001). Like astrocytes, cerebromicrovascular endothelial cells also elongate and desaturate the short chain fatty acids but final desaturation step is absent in them and they tend to cooperate with the astrocytes to synthesise DHA (Moore, 2001; Moore et al., 1990).

Although some reports suggest the presence of mRNA for \(\Delta-5\) desaturase and \(\Delta-6\) desaturase, both of which are required for the conversion of ALA to DHA, in both human and rat placental tissue, albeit at a very low level (Cho et al., 1999, Wadhwani et al., 2013), there is no strong evidence that placenta can synthesize DHA. The placenta may, instead, play a crucial role in mobilizing the maternal adipose tissue to actively concentrate and channelize the LC-PUFA to the fetus via multiple mechanisms (Haggarty, 2004). On the other hand, in the mouse fetal brain, \(\Delta-6\) desaturase is found to decrease dramatically (12-fold) up to
postnatal day 21 and remained nearly constant there after (Bourre et al., 1990). This is insufficient for fulfilling the requirement of DHA during development, thus relying mainly on maternal transfer of LC-PUFA. Another n-3 fatty acid EPA mainly mediate transport of DHA across the placenta. A number of fatty acid transport proteins or nutrient carriers have been identified in the placenta that facilitate transfer of free FAs to meet the increased demand of the fetus during gestation. EPA has been positively correlated with mRNA expression of all these membrane proteins (Larque et al., 2006). FABPpm, a specific placental Fatty Acid Binding Protein binds only LCFA was isolated and purified from human placental membranes (Campbell et al., 1994).

Another two fatty acid transporters, FAT/CD36, and FATP, has been demonstrated in human placenta which helped bi-directional flow of all types of free FAs, unlike FABPpm which is located on the maternal side of the placenta, regulating the unidirectional flow of maternal plasma LCPUFA to the fetus (Abumrad et al., 1999) (Fig. 4).

Thus, maternal plasma level of DHA should be higher during pregnancy and evidences suggest higher level of circulating estrogen level is associated with upregulated synthesis of DHA from vegetable precursors (Giltay et al., 2004). In human infants, all elongases/desaturases necessary for the conversion are active in the first week after birth (Salem et al., 1996). Newborn brain has high concentration of DHA than in utero brain, but strikingly, in the latter, more than 50% of the DHA is accredited in fetal adipose tissue and not in the brain (Haggarty, 2004; Cunnane, 2005). Within a few hours of birth, the stored

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**Fig. 4.** Theoretical model of fatty acid movement across placental tissue. LP, lipoprotein; LPL, lipoprotein lipase; NEFA, non-esterified fatty acid; FATP, fatty acid transport protein; FAT, fatty acid translocase; P-FABPpm, placental plasma membrane fatty acid binding protein; L-FABP, liver-fatty acid binding protein; H-FABP, heart-fatty acid binding protein; PPAR, peroxisome proliferator activated receptor; RXR, retinoid X receptor (from Clin. Nutr. 2010, 27, 685–693)
DHA in adipose tissue begin to turn over at a high rate (Van Duyne and Havel, 1959) and this is largely used up in the first two months of postnatal life for brain and retinal development if the diet is devoid of preformed DHA (Farquharson et al., 1993). That the FA supply during the fetal and infant period is important for the optimal development of the brain and visual system was established in studies in non-human primates, which reported impaired cognitive function and visual acuity in offspring of mothers who were deficient in n-3 PUFA that could not be rescued by post-natal n-3 LC-PUFA supplementation even though n-3 LC-PUFA status was improved (Neuringer et al., 1986; Anderson et al., 2005). The concentration of ALA in the new-born (0.3%) is half that in the mother (0.6%), whereas DHA concentration is double (3% versus 1.5%) (Al et al., 1990; Hornstra et al., 1990; Innis, 1991). This situation, in which the relative plasma concentration of ω-3 (mainly DHA) exceeds that of their precursors, is specific to the new born and is never observed in the adult. It is obviously an extremely favorable situation for the development of the new-born, especially at a time when there is a rapid development of the brain and retina. Babies accrue DHA into the CNS until about 18 months of age (Denomme et al., 2005; Szajewska et al., 2006). The period also coincides with the peak period of synapse formation and synaptic plasma membranes have been found to contain high proportions of DHA in the ethanolamine phosphoglycerides, ethanolamine plasmologen and phosphatidylethanolamine, as well as in the phosphatidylserine (PS) and can reach as high as 35% of fatty acids in the mammalian brain (Innis, 2005). Experimental studies on animals have shown that n-3 fatty acid requirements for the developing fetal and infant brain DHA can be met from the supplementation of ALA from mother’s diet (Arbuckle et al., 1993; Greiner et al., 1997). DHA levels in human milk increase with maternal DHA intake, either from foods or supplements (Brenna et al., 2007; Jensen, 1995; Innis, 1992; Innis, 2004; Innis, 2013; Kent et al., 2006; Koletzko et al., 1992) and clinical studies have shown that women with higher intakes of DHA give birth to infants with higher blood concentrations of DHA (Innis, 2004). From this perspective, a dietary source of DHA is expected to reduce the risk of neural tissue DHA insufficiency. Several studies have reported a positive relation between visual and neurodevelopment and DHA amounts in mothers’ milk in breastfed infants (Campoy et al., 2012; Gibson and Makrides, 2001; Jensen and Lapillonne, 2009; Makrides et al., 2010). Several clinical studies, addressing the effect of supplementing DHA, usually with AA, to infant formula on the neurological and visual function development of infants, have been reviewed (Campoy et al., 2012; Simmer et al., 2011).
8. Importance of DHA

Omega-3 fatty acids, such as DHA, have been shown to have multifaceted roles to promote good health. Chronic inflammation is thought to be one of the prime cause of chronic cardiovascular disease (CVD) including other metabolic diseases like T2DM (Type 2 diabetes mellitus) and obesity, severe autoimmune diseases like rheumatoid arthritis, multiple sclerosis, systemic lupus erythromatosus etc. DHA having anti-inflammatory effects improve cellular function through changes in gene expression profile resulted in a decreased expression of genes involved in inflammatory and atherogenesis-related pathways, such as nuclear transcription factor-κB (NF-κB) signaling, eicosanoid synthesis, scavenger receptor activity, adipogenesis, and hypoxia signaling (Bouwens et al., 2009). Treatment with DHA, significantly downregulated production of inflammatory cytokines TNFα, IL-1β, IL-6, INF-γ and nitric oxide metabolites reduces oxidative stress preventing damage to cells and tissues and upregulated protective IL-10 ex vivo (Olson et al., 2013; Mirshafiey and Mohsenzadegan, 2009; Ramirez-Ramirez et al., 2013). So DHA is successfully used to treat these diseases. While DHA has beneficial effect on the various systems of our body, the main importance of DHA lies in its role in brain development. DHA is as important in brain as calcium in bones. Research suggests that DHA may support the following:

8.1. Healthy pregnancy and length of gestation

DHA, are critical to fetal and infant central nervous system (CNS) growth and development. The time of the most rapid neural and retinal development occurs in the second half of pregnancy, mainly during the third trimester. So, supplementation of the maternal diet later in pregnancy with omega-3 fatty acids, especially DHA, was thought to be especially important. (Innis and Frisen, 2008; Denomme et al., 2005; Burdge, 2004; Jensen, 2006; Koletzko et al., 2007). During the last trimester, the fetus accrues about 50 to 70 mg a day of DHA (Clandinin et al., 1981; Innis, 2005, Kuipers et al., 2012) when both maternal DHA intake and circulating DHA concentrations are important determinants of fetal blood concentrations of DHA and cord plasma phospholipid levels (Innis and Friesen, 2008). During this period, vascular and especially neural growth are greatest in the fetus and DHA and AA concentration exceed the concentration of their precursors, ALA and LA respectively (Clandinin et al., 1980b; Uauy et al., 2000; van Houwelingen et al., 1996). In contrast, in rats, which are more immature at birth, accretion of DHA occurs with a pronounced spike during the last three days of gestation and continues through weaning (Green and Yavin, 1996; Kishimoto et al., 1965).
Both EPA and AA serve as precursors for biologically active compounds called eicosanoids. EPA is a precursor for the 3-series of PGs and produces PGE3 and PGI3, which promote relaxation of myometrium (Olsen et al., 1986; Olsen et al., 1992) to allow for the harboring of the pregnancy. AA serve as a precursor of the potent 2-series prostaglandins (PGs) E2 and PGF2 (associated with the initiation of preterm labor), and the vasoconstrictor thromboxane (TX)A2 (associated with preeclampsia, a condition in pregnancy characterized by high blood pressure, sometimes with fluid retention and proteinuria) (Olson, 2003; Malatyalioglu et al., 2000). As DHA, is not involved with eicosanoid formation rather it along with EPA competes with AA to reduce production of 2-series eicosanoids (Smuts et al., 2003; Jacobson et al., 2008), so a balanced intake of omega-6 and omega-3 fatty acids makes less inflammatory and less immunosuppressive eicosanoids good for health of pregnant women and preterm birth of baby (Endres et al., 1989).

8.2. Brain development

Since neurons tend to accumulate DHA, the latter is expected to have an inevitable role in neuronal development. *In vitro* studies have shown that DHA and AA affect proliferation and differentiation of embryonic multipotent neural stem/progenitor cells NSPCs (Sakayori et al., 2011). DHA and AA promoted the maintenance of the neurogenic NSPCs but had little effect on their differentiation. For gliogenic NSPCs, while DHA promoted the maintenance and neuronal differentiation, AA did not promote the maintenance but facilitated differentiation of the gliogenic NSPC cells into astrocytes. There are other reports that DHA promotes the proliferation and neuronal differentiation of cultured NSPCs generated from embryonic stem (ES) cells (He et al., 2009) and that DHA induces the neuronal differentiation of cultured embryonic NSPCs (Katakura et al., 2009; Kawakita et al., 2006; Ma et al., 2010). ω-3 PUFAs also affect NSPC cells *in vivo*. Oral supplementation of DHA promoted neurogenesis in the hippocampus of rats fed with a fish oil (FO) deficient diet over three generations (Kawakita et al., 2006). Immature neurons of the dentate gyrus were increased after feeding *n*-3 PUFAs-rich diet (Dyall et al., 2010). Feeding a ω-3 PUFAs-deficient diet to pregnant rats causes inhibition or delay of neurogenesis in the embryonic brains of pups (Bertrand et al., 2006). A number of studies suggests that DHA supplementation promote neurogenesis, neuritogenesis and neurite growth (both length and branching) in hippocampal neuron as well as cortical neuron in newborn as well as in adult (Boneva and Yamashima 2012; Calderon et al., 2004; Cao et al., 2005; Cao et al., 2009; He et al., 2009; Robson et al., 2010). DHA supplementation at low dosage (10-30 μM) to PC12 cell, induce mRNA levels of the neuronal differentiation markers like Egr1, Egr3, PC3 and PC4 genes. This induction
begins as early as 2 h post-DHA supplementation and continues throughout differentiation of PC12 cells (Dagai et al., 2009). During the brain development and membrane expansion, phospholipid doubling is an essential prerequisite and occurs in S-phase of mitosis (Jackowski et al., 1994; Jackowski et al., 1996). Deficiency of ω-3 fatty acid disturb their synthesis and turnover rate, brain unesterified DHA are not detectable and DHA incorporation to membrane from plasma is reduced by 40 fold and thus DHA content in different phospholipid classes become reduced by 83-88% in ω-3 deficient rats (Contreras et al., 2000). Studies on the effect of DHA on rat cortical neuron culture indicate that the increase in neuron viability and growth is concentration dependent, maximum effect being at 25 μM DHA, whereas higher concentrations (100-200 μM) of DHA cause reduction in cell viability (Cao et al., 2005).

Another important aspect of neuronal development is the migration of multiplying neurons from the sub-ventricular zone to their final destination in the various brain layers. Deficiency of ALA, during pregnancy is reported to cause transient decrease in neuronal migration to cortical plate during brain development (Yavin et al., 2009). Non-migrating cells remained confined in cortical laters IV-VI, corpus callosum and in sub-ventricular zone. A delayed migration of neuronal cells to CA1 and dentate gyrus where cells were retained in subicular zone was also observed under similar conditions (Brand et al., 2010a; Yavin et al., 2009). This delay in cell migration could be due to reelin (a glycoprotein important for proper cortical lamination) deficiency or disorganization which could affect the formation of lateral connections between neurons in the later stage and thus hamper in adult brain function (Yavin et al., 2009). Open pilot studies on children suffering from dyslexia, a migratory disorder, suggest that daily DHA rich supplementation significantly improved some of their learning skills like word decoding (speed of reading) and letter decoding (motoric-perceptual speed) abilities (Lindmark and Clough, 2007). Dark adaptation shown to be impaired during dyslexia improved after supplementation with a DHA–rich FO (Stordy, 2000).

In embryonic hippocampal neuronal cultures, DHA induce neurite growth, synaptogenesis, synapsin, and glutamate receptor expression (Cao et al., 2009). Oral administration of uridine and DHA, the circulating precursors of brain phosphatides was observed to increase in the synapse resulting in dendritic spine formation in rodents (Wurtman et al., 2009) and garbil (Sakamoto et al., 2007). This increase in synapse number, in turn, increases brain levels of synaptic proteins as PSD-95, synapsin-1, syntaxin-3 and F-actin, but not the levels of non-synaptic brain proteins like beta-tubulin. The ability of AA to activate syntaxin 3 which can partner with other SNAREs during membrane expansion of growth cones can be efficiently substituted with DHA and ALA (Darios and Davletov, 2006).
Such evidence highlight the relevance of omega-3 PUFAs in the various stages of brain development such as neuronal regeneration and neurite outgrowth which correlates closely with activation of syntaxin 3.

DHA has profound effect on neurotransmission. It can regulates membrane fluidity which in turn supports membrane protein functions impacting on speed of signal transduction and neurotransmission (Horrocks and Farooqui, 2004; Innis, 2007; Luchtman and Song, 2013; Parleeta et al., 2013a; Tassoni et al., 2008). DHA is released from the membrane by PLA2 and acts as an important intercellular messenger (Grintal et al., 2009). Dietary ω-3 FA deficiency significantly alters dopaminergic and serotonergic signaling in the fetal brain. Chronic dietary deficiency of ALA during various stage of development, from birth to 21 days of age induced changes in the synaptic levels of 5-HT, with higher levels of basal 5-HT release and lower levels of 5-HT-stimulated release by fenfluramine during adulthood (Kodas et al., 2004). Long-term feeding of ALA-deficient diet caused significantly higher 5-HT2 receptor density in the frontal cortex without affecting the endogenous serotonin concentrations when compared with normal diet fed controls (Delion et al., 1994). Serotonin concentrations in the brain of new born offspring from dams, fed throughout gestation with diet deficient in ALA, was found to be significantly higher compared to those from dams fed with diets rich in DHA (Innis and de la Presa Owens, 2001). On the contrary, dopamine concentrations were found to be lower in the offspring receiving low levels of ALA in the above study. Such decrease in dopamine has also been observed by others during ω-3 FA deficiency (Delion et al., 1994). Studies on D2 receptor density in various brain regions due to dietary ω-3 FA deficiency showed significant decrease in frontal cortex whereas in other regions exhibited increased receptor density (Delion et al., 1994; Kuperstein et al., 2005; Zimmer et al., 2002). In another study dietary depletion of PUFA during early development was found to cause a marked decrease in tyrosine hydroxylase with a concomitant down-regulation of the vesicular monoamine transporter (VMAT-2) and a depletion of VMAT-associated vesicles in the hippocampus (Kuperstein et al., 2008).

In addition to neurons, DHA contributes significantly in glial cell development and function. DHA facilitates astorcytes differentiation in vitro (Joardar et al., 2006). It essentially favour the coupling of gap junction in astrocytes, important for neuron-glia interaction required for proper brain development. Basically it enhances the phosphorylated isoform of connexin-43, main gap junctional protein expressed in astrocytes and radial glial cells and also cause redistribution of functional connexin-43 (Champeil-Potokar et al., 2006). Cortical glucose uptake and metabolism has been found to be DHA sensitive (Pifferi et al., 2005, Ximenes da Silva et al., 2002). MRI studies on human also show adequate levels of
DHA have the same involvement (Giedd et al., 1999, McNamara et al., 2010) in human cortical astrocytes development.

On the other hand, a recent study suggest DHA to cause suppression of microglial activity by inhibiting the PPARg/NF-κB pathway by directly affecting the gene transcription of TNF-α, IL-1β, ICAM-1, RAGE (receptor for advanced glycation end product) produced by microglial activation (Wang et al., 2015). Imbalance in n-3 PUFA in perinatal period alter microglial phenotype and motility and increase pro-inflammatory cytokines expression (Madore et al., 2014), further substantiating a putative role of n-3 PUFA in the management of infection or inflammation in the developing brain.

It has been shown that a 15-week diet deprivation of DHA reduces BDNF expression in the frontal cortex (Rao et al., 2007b). Alternatively, DHA dietary supplementation has been found to elevate levels of hippocampal BDNF, as well as AKT and CaMKII, indicating that DHA modulates mechanisms involved in synaptic plasticity (Wu et al., 2008). Likewise, rats fed for 15 weeks with a DHA enriched diet showed a significant increase in Fos-positive neurons in CA1 region of hippocampus (Tanabe et al., 2004) indicating that DHA can mediate mechanisms involved in synaptic plasticity and memory (Bousquet et al., 2009; Venna et al., 2009).

PUFAs are important components of myelin. Although myelin has its characteristic lipid composition containing mainly oleic acid and stearic acid, DHA constitutes only about 1.4% (Bourre et al., 1984). It has been shown that maternal dietary supplementation can significantly increase the DHA content of myelin in rat pups (Haubner et al., 2007). Reports indicate that ω-3 PUFs have significant role on the oligodendrocytes and myelin. In vitro studies have demonstrated that providing oligodendroglia cells with n-3 PUFAs, significantly increased their degree of differentiation (Van Meeteren et al., 2006). Hydrogen peroxide induced death of oligodendroglial OLN-93 cells can be significantly prevented by the addition of DHA and EPA (Brand et al., 2001) by the upregulation of heme oxygenase-1 (Brand et al., 2010b). In vivo studies, injecting a single dose of EPA or DHA intracerebroventricularly in 2 day old rat pups, stimulated the expression of myelin specific proteolipid protein, myelin basic protein and myelin oligodendrocyte protein mRNAs in various regions of the brain, the effect was more pronounced in EPA-treated rats (Salvati et al., 2008) suggesting a profound role of the ω-3 PUFs in the process of myelinogenesis. 2′-3′-cyclic nucleotide 3′-phosphodiesterase protein, an indicator of accelerated myelination, also increased. ω-3 PUFs have been found be beneficial in various types of myelin pathology like multiple sclerosis (MS), spinal cord injury (SCI), certain peroxisomal disorders etc. However, most of these studies have been carried out in the adult, both human and
experimental animals (Weinstock-Guttman et al., 2005; Jelinek et al., 2013, King et al., 2006; Kong et al., 2011; Huang et al., 2007; Martinez and Vazquez, 1998). However, one study using microglia cultures from new born rat pups showed that the activation of microglial phagocytosis by myelin or IFN-\(\gamma\), required for inducing the processes of demyelination, can be inhibited by the addition of DHA or EPA, both of which possibly inhibit the release of nitric oxide and TNF-\(\alpha\) (Chen et al., 2014).

8.3. Visual development and function

Visual development is incomplete at birth; maturation of the visual system-including neurological and ocular components is influenced by many factors including prenatal and postnatal nutrition and postnatal visual stimulation. Visual capabilities in neonates are dependent on the development of visual pathways in the visual cortex and lateral geniculate nucleus (LGN) in the thalamus. The LGN is immature at birth and a rapid increase in postsynaptic surfaces is seen in the first few months after birth. Synaptogenesis in the cortex is also rapid after birth, with a maximum synaptic density at about 8–9 months. Postnatal structural eye development includes maturation of the macula region of the retina, maturation of the fovea, and eyeball growth (Garey, 1984; Teller, 1982). DHA are found in the phospholipid layer of neuronal and retinal membranes and are thought to play a role in retinal and visual cortex maturation (Morale et al., 2005). In early life, particularly during sensitive periods of development, nutritional deprivation or preterm birth is thus associated with a high risk of visual impairments such as oculomotor and refractive abnormalities (Birch and O’Connor, 2001). Supplementation of DHA protect preterm birth and also nonbreast-fed term infants against visual development abnormalities. Retinal rod outer segments obtained from rats raised n-3 deficient rats led to functional deficits in each step in the visual signaling process (Mitchell et al., 2012). During development of the mammalian retinocollicular pathway, a reduction in DHA levels delays transitory misplaced axonal elimination. Disturbances in the axonal reorganization during the critical postnatal weeks interfere with the maintenance of terminal fields in the visual system (de Velasco et al., 2012). DHA has a strong influence on the proper development and survival for retinal photoreceptor cells. Deficiency in DHA in culture leads to extensive damage of cells by apoptosis. DHA supplementation delayed apoptosis by preventing mitochondrial damage, promote differentiation of cells, increase opsin protein expression (Politi et al., 2001). Mullar cells can take up DHA and incorporate it in the glial phospholipid and channelized it to photoreceptor cells where it acts as a trophic factor. Additionally, DHA gives protection to eyes, being
involved in tear formation and lacrimal and meibomian gland function (Harauma et al., 2014).

8.4. Cognition and memory
In rat, micronutrients imbalanced diet during pregnancy, affect the brain DHA and neurotrophins level at birth that again create cognitive deficit in later stage. Release of DHA by iPLA2 is a critical facet of corticostriatal LTP and depotentiation (Mazzocchi-Jones, 2015) and thus provides an exciting cellular target for the positive facilitation of cognitive function observed following DHA dietary supplementation (Mazzocchi-Jones, 2015). Reduced DHA intake and blood DHA are associated with age-related cognitive decline and Alzheimer's disease (Yurko-Mauro, 2010). Another study consisting of 148 patients with cognitive impairment [Mini-Mental State Examination (MMSE) score <24] and 45 control patients (MMSE score ≥24) showed that serum cholesteryl ester-EPA and -DHA levels were significantly lower (Tully et al., 2003). Image analysis of brain sections of an aged Alzheimer’s disease (AD) mouse model showed that overall plaque burden was significantly reduced by 40.3% in mice with a diet enriched with DHA. The largest reductions (40–50%) were seen in the hippocampus and parietal cortex that are thought to be involved with AD (Lim et al., 2005). A central event in AD is thought to be the activation of multiple inflammatory cells in the brain. Release of IL-1B, IL-6, and TNF α from microglia cells may lead to dysfunction of the neurons in the brain (Freund-Levi et al., 2009). In a study, AD patients treated with EPA+DHA supplementation increased their plasma concentrations of EPA and DHA, which were associated with reduced release of inflammatory factors IL-1B, IL-6, and granulocyte colony–stimulating factor from peripheral blood mononuclear cells (Vedin et al., 2008). Although there are conflicting data regarding the use of omega-3 fatty acids in terms of cognitive function, still results from studies about the positive effect of DHA regarding the disease processes of AD seem to be promising. Studies also revealed that Fish consumption is associated with reduction in AD risk in Americans and French adults (Barberger-Gateau et al., 2002; Morris et al., 2003). Raji et al. observed that weekly fish consumption was positively associated with gray matter volumes in several sub-structures including hippocampus and cingulated and orbitofrontal cortices (Raji et al., 2014).

Studies conducted in school children after feeding DHA or DHA enriched diet showed considerable improvement in learning and memory (Dalton et al., 2009), reading (Dalton et al., 2009; Richardson et al., 2012), spelling (Dalton et al., 2009), non-verbal cognitive development (Parletta et al., 2013b) and processing speed, visual perceptive capacity, attention and executive function (Portillo-Reyes et al., 2014). Supplementation with the FO,
Cod liver oil containing a large amount of DHA from 18 weeks of gestation to 3 months after delivery results into high score in the Mental Processing Composite of the K-ABC (measure of intelligence in children aged more than 2.5 years) at 4 years of age that is it is favourable for later mental development (Helland et al., 2003). Studies on laboratory animals corroborate the clinical findings (Luchtman and Song, 2013). Artificial rearing of mouse pups on a ω-3 FA deficient diet not only resulted in 51% loss of total brain DHA but also caused impaired learning in the reference-memory in the Barnes circular maze (Fedorova et al., 2007). However, locomotor activity in the open field test or in anxiety related behaviour in the elevated plus maze did not differ between these deficient mice and the ω-3 FA adequately fed controls. DHA fortification, considerably enhanced reference and working memory performance in animals (Chung et al., 2008). Considering that DHA is resistant to depletion, having a long half-life of ~2.5 years in human brain (Umhau et al., 2009), an appropriate study was undertaken where ω-3 PUFA depletion was carried out over successive generations. Results showed decreased brain DHA level, in the frontal cortex and hippocampus areas as well as impaired brain functions including changes in learning, memory, auditory and olfactory responses (Moriguchi and Salem, 2003). These effects were, however, restored by repletion with dietary DHA. DHA has been shown to accumulate in areas of the brain associated with learning and memory, such as the cerebral cortex and hippocampus (Chung et al., 2008; Gamoh et al., 1999). DHA deprivation caused marked decreases of synapsins and glutamate receptor subunits with concomitant impairment of long-term potentiation, a cellular mechanism underlying learning and memory. In the transgenic fat-1 mice, rich in endogenous n-3 PUFA, increased hippocampal neurogenesis due to greater number of proliferating neurons and increased density of dendritic spines of CA1 pyramidal neurons has been reported (He et al., 2009). These fat-1 mice also exhibited a better spatial learning performance in the Morris water maze compared with control WT littermates. Studies in healthy pups from healthy dams treated with ω-3 FAs during pregnancy and breast-feeding period showed improved dentate gyrus-LTP and enhanced Morris water maze performance (Kavraal et al., 2012). A marked reduction in the mRNA levels of memory related proteins, BDNF, NGF, TrkB, and CREB was recorded, which can be restored by prenatal EPA and DHA supplementation (Sable et al., 2012; Sable et al., 2014).

8.5. Prevention of cancer
Contrary to its beneficial effect on normal cells, several reports suggest that ω-3 FA, particularly DHA, negatively influence cancer cell proliferation (Chamras et al., 2002). Exposure of DHA to cancer cells induce apoptosis (Jacobsen et al., 2008; Sun et al., 2011),
promote differentiation (Chamras et al., 2002), both in vivo and in vitro. In tumor cells, DHA inhibits angiogenesis (Spencer et al., 2009), cell invasion (D’Eliseo et al., 2012). For these reasons, DHA is being increasingly used as an adjuvant in cancer chemotherapy, with particular emphasis to its capability, both to enhance the uptake of anticancer drugs, especially in cells otherwise resistant to these drugs, and to increase the pro-oxidant and proapoptotic efficacy of some chemotherapies (Siddiqui et al., 2011). DHA improve tumor cell cytotoxicity by modulating redox status of cells, inducing lipid peroxidation and oxidative stress in tumor cells.

8.6. Prevention of the occurrence of neurodevelopmental and neurodegenerative diseases

Since DHA plays an essential role in the various stages of brain development, inadequate intake through nutrition, results in a number of developmental disorders. Additionally, DHA is well known for its anti inflammatory mode of action in various tissues. Pure EPA and DHA is known to inhibit the production of many inflammatory proteins (Draper et al., 2011; Kong et al., 2010; Lee et al., 2001; Lo et al., 1999; Wellhauser and Belsham, 2014). Since the etiology of many of the developmental neurological disorders like Autism Spectrum Disorder (ASD), Attention Deficit Hyperactivity Disorder (ADHD) involve inflammatory pathology, it was natural to investigate any beneficial role of DHA in these disease conditions. Alteration in fatty acid content and phospholipid metabolism may also involve a number of developmental complicacies in ASD, ADHD, dyslexia, dyspraxia etc. A number of psychiatric disorders have also been found to have an effective correlation with deficiency of DHA. Hence, supplementation of ω-3 fatty acids is being encouraged in improving the therapeutic efficacy of antidepressants, mood stabilizers, antipsychotic drugs. In addition to alterations in TCA energy production, ammonia detoxification and abnormal cholesterol metabolism, there is reduced synthesis of omega-3 DHA in ASD (Aneja and Tierney, 2008; Gorker and Tuzum, 2005; Frye, 2014; Park et al., 2014). Clinical studies showed that supplementing omega-3 fatty acids for 6 weeks resulted in improvement in the aberrant and stereotypic behaviour of autistic children (Amminger et al., 2007). High dose of DHA could improve the social interaction in autistic children by increasing the plasma levels of SOD and transferrin (Yui et al., 2012). DHA has beneficial role in ADHD, a syndrome of inattention, impulsivity, and/or hyperactivity, mostly diagnosed in children. Eight week supplementation with EPA and DHA decreased plasma C reactive protein (CRP) and IL-6 level and increase SOD and glutathione reductase activity in ADHD children, thereby decreasing the plasma inflammatory mediators and oxidative stress to reduce neuroinflammation (Hariri et al.,
Similar dose regime used to assess behavioral studies in ADHD showed improvements in behaviors paradigms like inattention, hyperactivity, oppositional/defiant behavior and conduct disorder (Sorgi et al., 2007). Several abnormalities in cortical maturation and development of attention occur due to prenatal DHA deficiency (Colombo et al., 2004; Helland et al., 2003; Judge et al., 2007; Kodas et al., 2002; Levant et al., 2004; McNamara and Carlson, 2006) are observed in patients with schizophrenia (Brown et al., 1996). Mothers with elevated serum DHA levels also increased the risk of schizophrenia in the offspring (Harper et al., 2011). Supplementation of DHA prevented lipid damage in hippocampus and striatum, protein damage in prefrontal cortex, also inhibited startle reflex (Zugno et al., 2014). ω-3 supplementation also reduced acetyl choline activity in prefrontal cortex, hippocampus and striatum of ketamine induced schizophrenic model of Winster rat (Zugno et al., 2015).

DHA is also helpful in preventing several neurodegenerative diseases. AD brain exhibit reduction in DHA and its oxidative derivative NPD1 (Lukiw et al., 2005). Various markers of AD neuropathology are sensitive to n-3 PUFA in the CNS. A high n-3 PUFA content in brain tissue is associated with reduced Aβ levels (Lebbadi et al., 2011; Lim et al., 2005), reduced soluble hyperphosphorylated tau (Lebbadi et al., 2011; Green et al., 2007), higher synaptic marker levels (Calon et al., 2004; Calon et al., 2005; Perez et al., 2010) and improved cognitive performance (Calon et al., 2004; Hooijmans et al., 2009) in animal models of AD. DHA suppresses both Aβ40 and Aβ42 peptide release with concomitant NPD1 synthesis which inhibits Aβ42-induced apoptosis in HN (Human neural progenitor cells) cells. In case of Parkinson’s disease (PD), DHA inhibits the nuclear translocation and activity of NF-κB p65 protein which is associated with COX-2 expression and prostaglandin E2 production (Vijitruth et al., 2006) in dopamine-containing neurons of the substantia nigra pars compacta region of the CNS. This inhibition of COX-2 by DHA (Lee et al., 2009) subsequently inhibit inflammatory signaling pathways mainly involved in dopaminergic neuronal cell death in PD (Cansev et al., 2008). In addition, DHA protects dopaminergic neurons by enhancing the expression of glial-derived neurotrophic factor (GDNF) and neurturin in MPTP-induced Parkinsonism (Tanriover et al., 2010). Retinal degeneration leads to apoptotic death of photoreceptor cells. DHA is retained and protected from peroxidation in the retinal photoreceptor cells. Oxidative damage to DHA produce trans-4-hydroxy-2-hexenal (HHE) which is toxic to neurons, depletes GSH and increases reactive oxygen species (Long et al., 2008) causing cell death. Decreasing the amount of the major target of lipid peroxidation (DHA) contributes to the protection of photoreceptors (Anderson et al., 2002). Although a decrease of DHA content in blood has been reported in the degenerative...
diseases of the eye, the relationship between decreased DHA in the blood supply and disease initiation and progression is yet to be understood.

9. ω-3 Fatty Acids and gene expression

DHA is the precursor of several bioactive products, because it can be converted via enzymatic and non-enzymatic oxidation. DHA can be metabolized into e.g., resolvins D1–D5, maresin-1 and neuroprotectin D1 by means of enzymatic oxidation. In non-enzymatic oxidation, DHA can be oxidized by radical oxygen species into lipid peroxides such as neuroprostanes, isoprostanes, aldehydes and 4-hydroxyhexenal. These peroxides can activate transcription factors such as peroxisome proliferator-activated receptors (PPARs) and nuclear factor-like 2 (Nrf2). These transcription factors affect the expression of several key proteins pertinent to inflammation, lipid metabolism, and energy utilization. (Table 3)

Table 3: Genes influenced by n-3 fatty acids.

<table>
<thead>
<tr>
<th>Inflammatory proteins</th>
<th>Energy/ Lipid metabolism</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF-κB, IKKs, iNOS, IFNγ, IL-1b, IL-2, IL-6, IL-8, IL-12, E-selectin, ICAM1, VCAM1, MCP1, MMP9, TNF-α, COX-2</td>
<td>PPAR-α, PPAR-γ, PPAR-δ, SREBPs, aP2, ACO, Leptin, PDK-4, apoE</td>
</tr>
</tbody>
</table>

Key representative genes critical for inflammation and lipid metabolism are listed. In general, proinflammatory genes (left column) are suppressed by n-3 fatty acids, whereas genes critical for lipid peroxidation, energy utilization, and lipid homeostasis are increased by n-3 fatty acids (right column) (from Am J Clin Nutr. 2006, 83(suppl):1520S–5S.)

However, in different tissues, n-3 fatty acids can up-regulate or down-regulate the expression of different proteins (Lee and Hwang, 2002). For example, a diet enriched in n-3 fatty acids reduces peroxisome proliferator-activated receptor-α (PPARα) expression in adipose tissue without affecting PPAR-γ expression. In contrast, an unpublished observation cited in a report suggest that the expression of both PPARα and PPARγ is induced in the arterial wall in an insulin resistant mouse model (Deckelbaum et al., 2006). Possibly expression of various intermediates such as transcription factors, nuclear hormone receptors, and lipid second messengers, trigger a cascade of other genes or other effects in the cell that then affect gene expression (Fig. 5) Direct molecular interaction of n-3 fatty acids with certain genes is also possible but literature support is rare.
10. Mode of action

FAs are used only poorly as fuel in the brain (Schonfeld and Reiser, 2013) and therefore, used for other biological functions. There are four general mechanisms by which (n-3) PUFA could affect cell and tissue behavior to elicit their physiological actions:

1) n-3PUFA could influence metabolite and/or hormone like inflammatory signalling molecules concentrations that in turn influence cell and tissue behaviour. Via these indirect mechanisms, DHA mediate anti-inflammatory effects on cells. During tissue stress, both EPA and DHA may be released from phospholipids and oxidized to potent signaling molecules, resolvins and protectins, collectively known as docosanoids by LOX enzyme. It has been recently shown that the DHA metabolite, with an endocannabinoid-like structure, N-docosahexaenoylthanolamide (DEA) or synaptamide, has a key contribution towards hippocampal neuronal development, synaptogenesis and synaptic function (Kim et al., 2011a; Kim et al., 2011b). It promotes neuronal differentiation and is a much more potent promoter of neurite growth, synaptogenesis and synaptic function than DHA itself (Kim et al., 2011a; Kim and Spector, 2013). The brains of mice that are fed with high levels of DHA have increased synaptamide levels and show increased neuronal differentiation of neural stem cells (Rashid et al., 2013). Another metabolite, Neuroprotectin D1 (NPD1), a derivative of DHA and normally present in the brain is synthesized by LOX. NPD1 promotes neuronal survival by upregulating genes encoding various anti-apoptotic proteins and down-regulates genes encoding the pro-apoptotic proteins in vitro and also in the brain in vivo (Lukiw et al., 2005;
Lukiw and Bazan, 2008; as reviewed in Bazinet and Laye, 2014). Its anti-inflammatory effect mainly mediated by inhibiting the NF-κB.

2) n-3PUFA could influence other factors (e.g., oxidation of LDL; oxidative stress) that in turn influence cell and tissue behaviour.

3) n-3PUFA could have direct effects on cell behaviour via surface or intracellular fatty acid “receptors” or “sensors”. During the past decade, a number of studies revealed that free FAs also act as key signalling molecules to regulate a number of physiological functions through G-protein-coupled receptors (GPCRs) of which GPR40 and GPR120 are established as putative receptors for DHA and EPA (Miyauchi et al., 2009; Oh et al., 2010). Although GPR40 and GPR120 are widely detected in the various brain areas, GPR40 has been more extensively studied in the brain. Over-expression of GPR40 in rat neural stem cells and in PC12 cells promoted neurogenesis via the activation of PLC/IP3 signalling and intracellular Ca++ mobilization (Yamashima 2008; Ma et al., 2010). An interaction of PUFA-GPR40-pCREB in adult primate neurogenesis has been reported (Boneva and Yamashima, 2012). The inhibitory effect of DHA on NF-κB occurs via GPR120 involving anti-inflammatory signaling.

In addition, cell based assays, for the first time, identified DHA to be an intrinsic ligand for the activation of RXR receptors in brain (de Urquiza et al., 2000). Subsequent studies confirmed direct binding of DHA to RXR, in addition to other FAs (Lengqvist et al., 2004; Stafslien et al., 2007). Many of the effects of DHA on mood and memory have been recently reported to be suppressed by antagonist of RXR while pan RXR agonist show DHA mimicking effects (Wietrzych-Schindler et al., 2011). In a recent study, the 7TM adiponectin receptor 1 (AdipoR1) to which the hormone, adiponectin binds has been found to be a key regulator of photoreceptor cell survival through DHA uptake, retention, conservation and elongation to VLC-PUFAs (Rice et al., 2015). The opening of background K(+) channels, like TREK-1 and TRAAK, which are abundant in the brain and located at both pre- and post-synapse are specifically activated by AA and other PUFAs such as DHA and LA, unlike the saturated fatty acids (Lauritzen et al., 2000).

4) n-3PUFA affects cell behaviour by changing the composition of cell membrane phospholipids. Contrary to the belief that presence of very long chain long 22 carbons in DHA rich membranes would cause the membranes to be exceptionally thick, actually these are quite thin due to DHA’s unusual three dimensional conformation (Dratz et al., 1985). DHA undergoes rapid inter-conversions between multiple torsional states, conferring high membrane permeability, compression, fusion, and flipflop rates (Hasadsri et al., 2013). Such versatility of DHA to alter the biological properties of cell membranes helps downstream
signaling events, promote gene activation, neurotransmitter release, oxidative stress, or to regulate mitochondrial physiology, thereby governing various cellular functions (Chalon et al., 1998; Sergeeva et al., 2005). In microglia, exogenously added DHA, is specifically enriched in membrane phospholipids, but not in raft lipids of microglial cells. Such incorporation of DHA in membrane has been shown to inhibit lipopolysaccharide receptor presentation on cell surface but not in its membrane subdomain localization. Lipid rafts, subdomains of cell membranes, are potentially modifiable by dietary n-3 fatty acids. Although dietary PUFA are not incorporated into raft lipids because of their low affinity to cholesterol, they are incorporated into non-raft regions and seem to influence raft formation and function, displacing key signaling proteins and altering intracellular protein trafficking (Chapkin et al., 2008; Fan et al., 2003; Fan et al., 2004; Kim et al., 2008; Rockett et al., 2011; Shaikh et al., 2009; Sidhu et al., 2011; Stulnig et al., 2001; Zeyda et al., 2002; Zeyda et al., 2003). Studies indicate that by modifying protein and lipid organization of the plasma membrane lipid raft structure, DHA essentially regulates essential cell signaling events (Mirmikjoo et al., 2001; Seebungkert and Lynch 2002; Stillwell and Wassall 2003).

Fig. 6. Overview of the mechanisms by which (n-3) PUFA can influence cell function. (from J. Nutr. 2012, 142:592S-599S).
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Rationale of the present study

It is amply clear that DHA is an important nutraceutic, having profound role in the development of the mammalian brain. It causes differentiation of neuronal as well as astrocytes. While it plays a critical role during the embryonic period, it is also beneficial for the treatment of several neurodevelopment and neurodegenerative diseases in adults. Thus DHA is protective for healthy brain cells, in general. At the same time, DHA is being increasingly used as an adjuvant in cancer chemotherapy, because of its ability to promote death in cancer cells. It is, therefore, interesting that a PUFA like DHA can play opposite roles in two different types of cells, being deleterious for neoplastic cells on one hand and promoting growth and differentiation of normal cell on the other. With a view to understand more about such varied mechanism of action of DHA, the present study was conceived, focusing on two areas.

Chapter-1: Identification of cytotoxic mediators associated with DHA-induced cell death in brain cancer cells, regulated differently in healthy primary astrocytes

The high throughput proteomic approach is widely used to analyze the alteration of protein expression, thereby allowing comparative analysis of proteome profile of different types of cells treated or non-treated with specific drug. Altered expression of proteins might cause abnormal cell signaling resulting in deviation from the normal cellular functioning and causing alteration in the cellular fate. For the present study, we have taken two cell lines, a) the rat glioma, C6G and b) the human neuroblastoma, SH-SY5Y representing neural cancer cells, in addition to primary culture of astrocytes representing healthy cells, and exposed them to DHA. We have first tested the occurrence of differential response of DHA in the two type of cells selected and then employed proteomics approaches to identify the common proteins which are differently affected in these two different types of cells upon exposure to DHA. Based on the information, attempt has been made to delineate the putative pathways leading to the cytotoxicity of the cancer cells.

Chapter - 2: Comparative studies on the molecular mechanism of Thyroid Hormone (TH) or DHA-induced astrocytes differentiation.

Astrocytes outnumber the neurons by several folds in the developed brain and provide structural, nutritional, bio-chemical support to the neurons, for their survival and growth. To achieve this, astrocytes undergo progressive changes in morphology during brain
development, each of which are required to carry out specific functions at the various periods of development. Much effort has been carried out to understand the mechanisms involved in such morphological changes of astrocytes during brain development and the intrinsic regulators vitally required for inducing such changes. Previous works from our laboratory as well as by others have established that TH strongly influence the morphogenesis of astrocytes. Using primary astrocyte cultures, it has been demonstrated that the immature radial glial cells (a no. of long processes giving star like appearance) are initially transformed into flat polygonal cells (having large cell body with single nucleus but without any processes) within 8-10 days. This is followed by a second or final transformation to single process bearing mature cells with stellate morphology (Paul et al., 1996). Under hypothyroid condition, the cells were arrested in polygonal state, and never achieve the final stellate morphology, unless supplemented with TH. In similar primary cultures, it has been demonstrated that DHA also comprehensively promotes astrocyte maturation (Joardar et al., 2006). Some mechanistic studies have identified downstream role of the β-AR system in both TH as well as DHA induced maturation of these astrocytes. The present study is a continuation of the previous observations with the aim to unravel the detailed mechanism by the above two regulators and provide a comparative analysis of their mechanism of action in promoting differentiation and maturation of astrocytes. The work carried out has been described in two parts followed by a comparative analysis.

Part-1: Identifying the key players during TH-induced differentiation of astrocytes

Part-2: Exploring the role of these key regulators in DHA-induced astrocytes differentiation