White Paper:

Omega-3 Fatty Acids: EPA/DHA

FATTY ACID CHEMISTRY 101

Fatty acids are made up of carbon, hydrogen and oxygen, joined together in chains of varying lengths (from 4-24 carbons).[1,2]

At one end of the chain is a methyl (or omega) group (CH3), and at the other is a carboxylic acid (or alpha) group (COOH).[1,2]

The chains can be classified as short, medium or long depending on how many carbons they contain. Short chain fatty acids (SCFAs) contain < 6 carbons; medium chains (MCFAs) contain 6-10 carbons and long chains (LCFAs) contain ≥ 12 carbons. They can also be saturated and contain no double bonds between carbon atoms or unsaturated and contain double bonds. The existence of double bonds means that not every carbon is surrounded by hydrogen atoms (hence, it is unsaturated).[2]

If a chain contains one double bond, it is considered a monounsaturated fatty acid (MUFA), if it contains more than one, it is a polyunsaturated fatty acid (PUFA).[3]

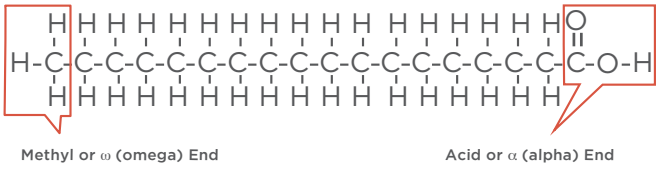


Figure 1: Structure of a saturated long-chain fatty acid[2]

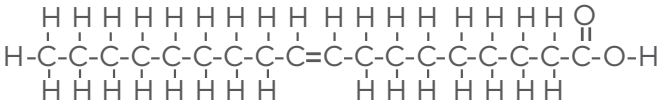


Figure 2: Structure of a long-chain monounsaturated fatty acid[2]

Unsaturated fatty acids can be further classified depending upon where in the chain, the double bond exists. For example, if the double bond occurs between the third and fourth carbon from the methyl end (or after the third carbon) it is classified as an omega-3 (ω-3) fatty acid. If it occurs between the sixth and seventh carbon (or after the sixth), it is an omega-6 (ω-6) fatty acid, after the twelfth carbon, an omega-12 (ω-12) and so on.[3]

There exist two fatty acids essential for humans – the ω-3 fatty acid alpha-linolenic acid (ALA) and the ω-6 fatty acid, linoleic acid (LA). Humans (and most mammals) have the biological ability to insert double bonds into most positions along the chain during fatty acid metabolism, with the exception of positions 3 and 6. Therefore, the body is unable to produce either ALA or LA using other fatty acids as precursors.[14]

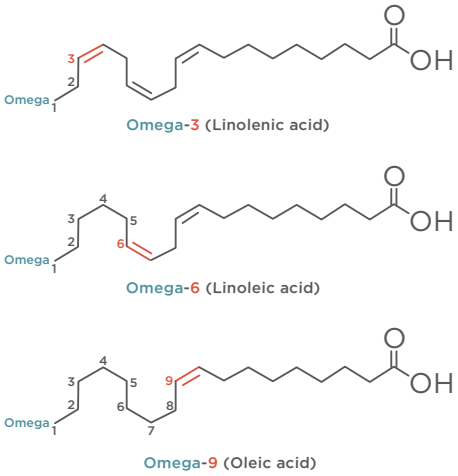


Figure 3: Structure of Omega-3, 6 and 9 fatty acids.

Omega-3 and 6 fatty acids both have biological activity and are required by the body for optimal functioning, hence these two fatty acids are considered essential for health and need to be consumed as part of a healthy diet. These two fatty acids are the parent/precursor compounds from which other omega-3 and 6 fatty acids vital to human health are synthesised.[4]

Nomenclature

Fatty acids are named based on the number of carbons and double bonds contained in the chain, and where in the chain the double bonds begin. For example, ALA has 18 carbons and 3 double bonds, with the first double bond occurring just after the third carbon (or in the ω-3 position), therefore, the correct nomenclature for ALA is C18:3 ω-3. LA also contains 18 carbons, but only two double bonds, with the first double bond occurring at the ω-6 position, therefore the correct nomenclature for LA is C18:2 ω-6.[14]

FATTY ACID METABOLISM

Fatty acid metabolism takes place largely in the endoplasmic reticulum of hepatocytes.[8] During this process, ALA and LA are lengthened and manipulated by both delta-5 and delta-6 desaturase enzymes resulting in a number of different compounds.

Delta 5 and 6 desaturase enzymes require several nutrients for optimal functioning. These include Vitamins B6, B9, B12, A and C, β-carotene and the minerals zinc, magnesium, and calcium. Deficiencies in any of these nutrients (particularly Magnesium, and B6) may hinder fatty acid metabolic processes resulting in impaired eicosanoid and prostaglandin production.[10]

LA & ALA

LA is metabolised down to Gamma-linolenic acid and further to arachidonic acid (ARA) which can enter pro-inflammatory pathways, but also has beneficial roles in the cell membranes and other cellular functions.[5]

ALA enters a metabolic pathway that results in the synthesis of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). Both of these ω-3 fatty acids have anti-inflammatory biological activity that is intrinsic to optimal human health.[8]

Both ALA and LA maintain ω-3 and ω-6 levels in specific tissues and are also responsible for the creation of both pro- and anti-inflammatory eicosanoids. They both compete with each other metabolically. High intakes of LA can inhibit the ALA conversion pathway, leading to the production and accumulation of series-2 pro-inflammatory eicosanoids (PGE2, PGA2, PGI2 and thromboxane A2). High intakes of ALA can encourage the production of series-3 anti-inflammatory eicosanoids including ω-3 PUFAs Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) which then accumulate in biological tissues and compete with the production of arachidonic acid (ARA) in the cyclooxygenase and lipoxygenase pathways.[8,14] This results in an inhibition of leukotriene synthesis and therefore inhibition of the production of inflammatory cytokines and C-Reactive Protein (CRP). It also causes an increase in the production of the anti-inflammatory series -3 prostaglandins, series-5 leukotrienes and D-series resolvins (D1 and D2), protectins (neuroprotectin D1) and maresins (MaR1 and MaR2).[8,14,15]

This leads one to the conclusion that a diet that favours high intakes of ω-3 fatty acids and low intakes of ω-6 fatty acids is preferable. Research suggests that consumption of ω-6 to ω-3 should occur in a ratio of 1:5-1:10, ideally mimicking Japanese diets which consist of a ratio of 1:2-1:4 to provide us with adequate dietary protection from inflammation and chronic disease.[3,8] In the West however, ratios of 15:1-16.7:1 are common and can lead to chronic inflammation, vasoconstriction and platelet aggregation, culminating in an increased risk of cancer and cardiovascular disease.[3,8]

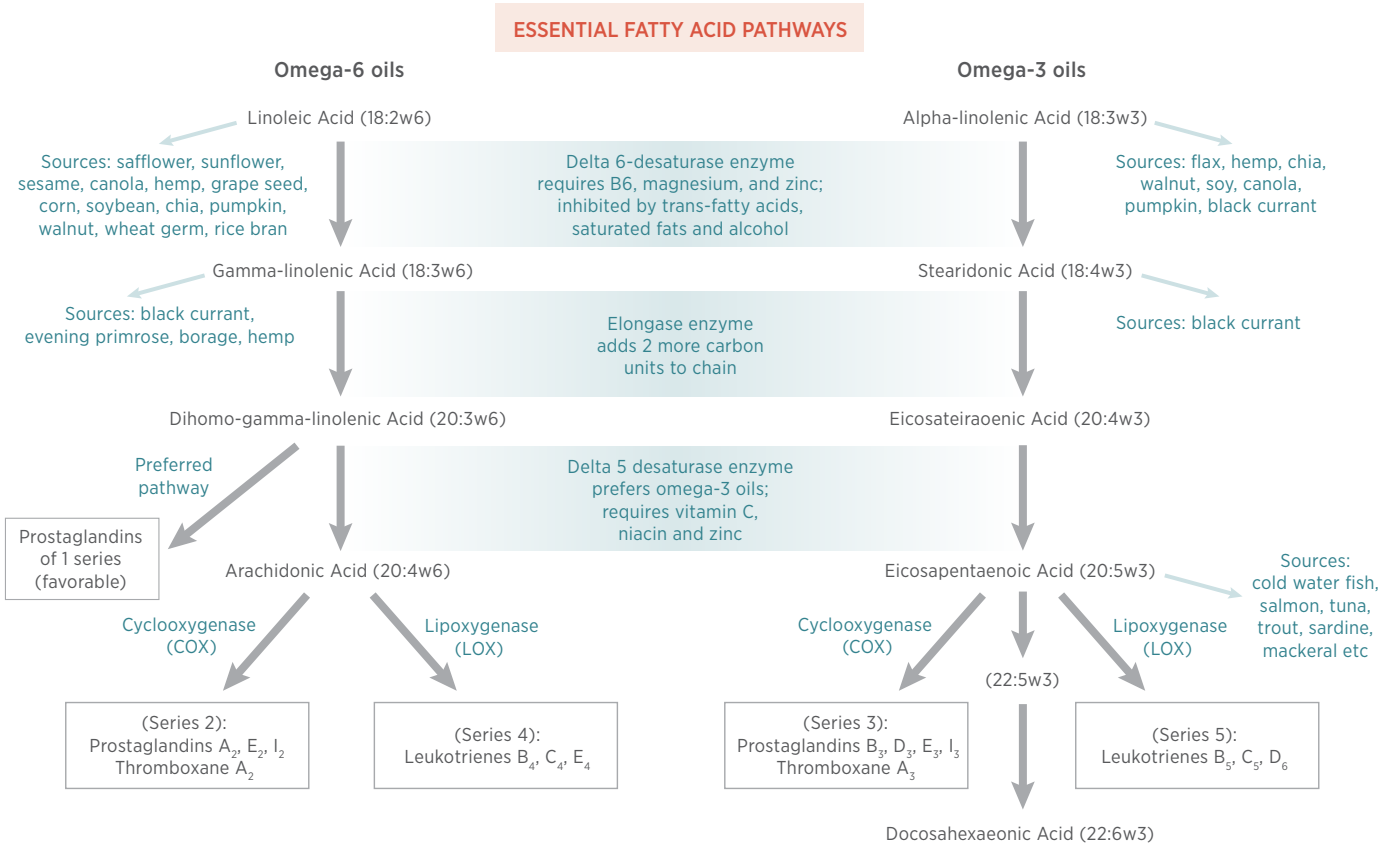
OMEGA-3 FATTY ACIDS

Omega-3 fatty acid pharmacokinetics

Upon ingestion, ω-3 PUFAs including ALA, EPA and DHA are hydrolysed in the intestines to monoglycerides and free fatty acids. These by-products incorporate into micelles that are then absorbed via passive diffusion into the enterocytes.[11,12]

Free fatty acids are then involved in chylomicron formation. Chylomicrons enter the circulation and are distributed throughout tissue to be metabolised or stored.[11,12]

Figure 4: Metabolic fatty acid conversion pathways for ω-3 and ω-6 fatty acids.[8,9]

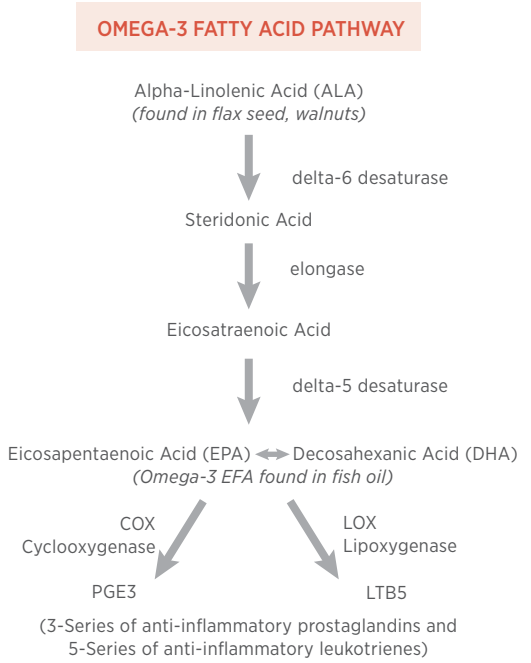


Metabolism of ω-3 PUFAs can take place via several processes, including beta-oxidation, enzymatic biotransformation, as well as the production of lipid mediators.[13]

Once in the circulation, ω-3 PUFAs are incorporated into the phospholipid cell membrane where, upon stimulation, are released by the phospholipase A2 enzyme and enter the fatty acid metabolic pathways for conversion into the longer chain eicosanoids, prostaglandins, prostacyclins and thromboxanes which go on to have influential biological effects in the immune, nervous and cardiovascular systems.[3,4]

When stored in the phospholipid cell membranes in the brain, ω-3 PUFAs contribute to the structure of the neuronal membrane and myelin sheath. When not stored in cell membranes, dietary fats are stored in fatty tissues until they are oxidised and enter the Kreb's cycle.[4]

Figure 5: Metabolic fatty acid conversion pathways for ω-3 polyunsaturated fatty acids.[7]



Omega-3 fatty acid metabolism

ALA supplements and food sources such as flax, chia, hemp and pumpkin seeds, walnuts, avocados and edamame may be consumed in order to ensure the production of EPA and DHA and gain the flow-on benefits of these two nutrients. However, this conversion pathway is rate-limited and can be inefficient. Only around 5-10% of ingested ALA ends up being converted to EPA, whilst only an average of 2-5% (sometimes as low as 0.25%) is converted to DHA.[4,8]

To bypass the inefficient conversion pathways, EPA and DHA supplements may be used or dietary sources such as deep-sea, cold-water fish, crustacea or organic chicken, beef or lamb can be consumed.

DHA – DOCOSAHEXAENOIC ACID (C22:6ω-3) & EPA - EICOSAPENTAENOIC ACID (C20:5ω-3)

Cell membrane fluidity

One of the primary biological roles of ω-3 fatty acids relates to membrane structure and fluidity[25] As membrane fluidity increases, so too does its biochemical and functional efficiency.[24] Cell membrane fluidity effects the function of receptors, membrane-bound enzymes, transport channels etc. Both EPA and DHA have a role in maintaining the fluidity of cell membranes, however DHA is the most fluidising of all compounds found in cell membranes (especially those of the brain, retina and spermatozoa), and adequate dietary intakes are vital for cell survival.[24]

Inflammation

As mentioned above, EPA and DHA can compete with ARA for inclusion in the cyclooxygenase and lipoxygenase pathways, thus inhibiting the production of pro-inflammatory leukotrienes. This inhibits the production of inflammatory markers including CRP and protein kinases such as JNK, MAPK and p38, as well as cytokines, chemokines and angiogenic growth factors including VEGF.[8,15] EPA and DHA can also inhibit PPARα receptors and the subsequent production of pro-inflammatory adhesion molecules such as ICAM-1 and VCAM-1.[8]

When EPA or DHA are used in this pathway instead of ARA, the production of anti-inflammatory eicosanoids such as lipoxins, resolvins, protectins and maresins results. These compounds can aid in the resolution of inflammation and enhance phagocytosis and immune function.[8]

DHA has particular anti-inflammatory role in the brain. DHA derived anti-inflammatory mediators including resolvins, maresins and neuroprotectins can help to prevent against neuronal ischemia and inflammation which has been suggested as a possible reason why there exists a negative correlation between depression and ω-3 PUFA intake.[8]

Figure 6: Eicosanoid production during fatty acid metabolism.[8]

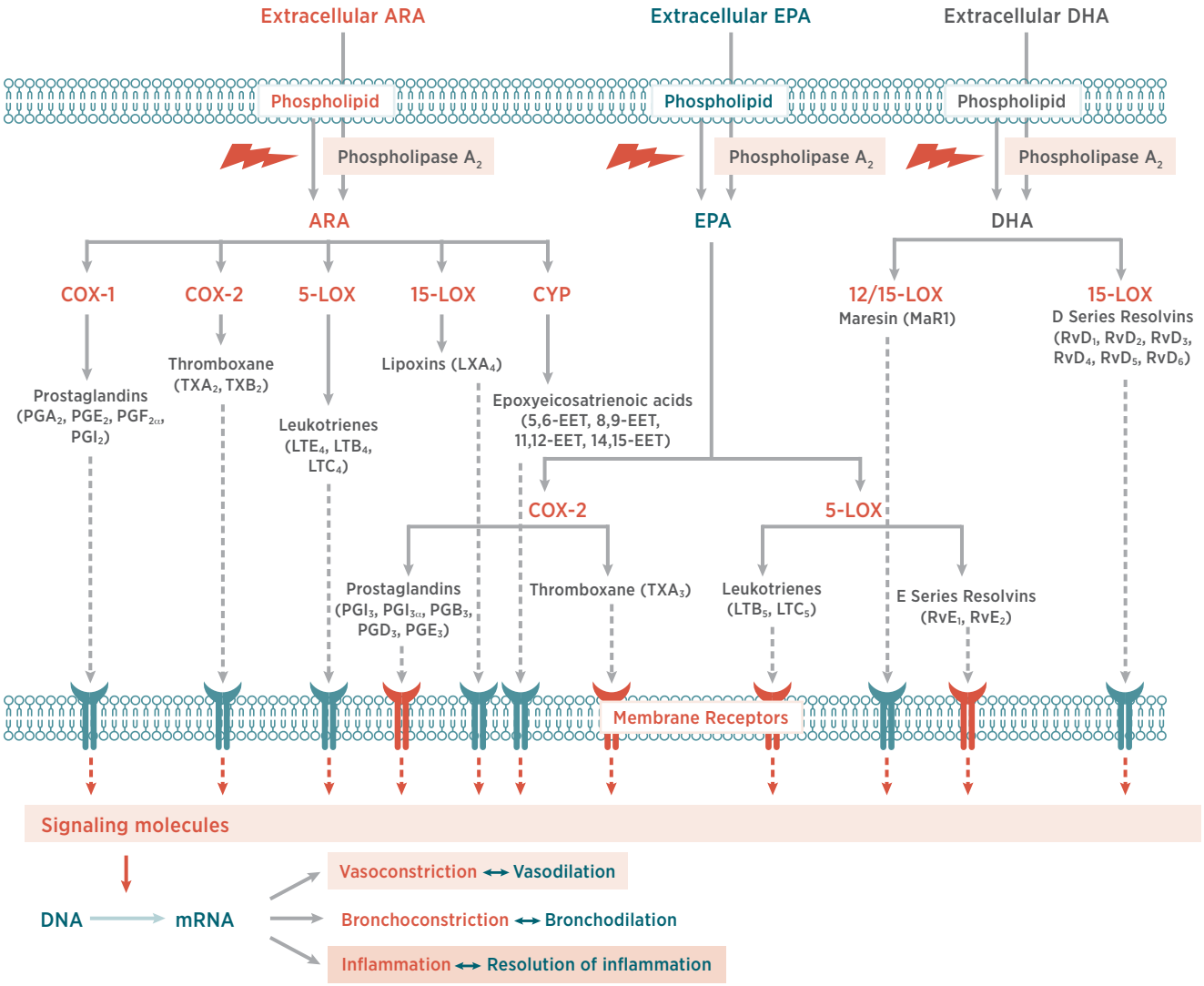


Table 1: Eicosanoid production during fatty acid metabolism.[8]

Enzyme	Arachidonic Acid (ARA)	Eicosapentaenoic Acid (EPA)	Docosahexaenoic Acid (DHA)
Cyclo-oxygenase-1	Series 2 Prostaglandins (PGA2, PGE2, PGF2, PGI2)		
Cyclo-oxygenase-2	Thromboxane (TXA2, TXB2)	Thromboxanes (TXA3)	
		Series 3 Prostaglandins (PGI3, PGB3, PGD3, PGE3)	
5-Lipoxygenase	Leukotrienes (LTE4, LTB4, LTC4)	Leukotrienes (LTB5, LTC5)	
		E-Series resolvins (RvE1, RvE2)	
15-Lipoxygenase	Lipoxins (LXA4)		D Series Resolvins (RvD1, RvD2, RvD3, RvD4, RvD5, RvD6)
12/15-Lipoxygenase			Maresin (MaR1)
CYP	Epoxyeicosatrienoic acids (5,6-EET, 8,9-EET, 11,12-EET, 14,15-EET)		

Cardiovascular system

A significant body of evidence has demonstrated an association between endogenous levels of EPA and DHA and cardiovascular health, which is attributed to a significant number of cardioprotective mechanisms.[26] EPA and DHA play a role in the homeostasis of cardiovascular health and function by supporting healthy blood lipid profiles, supporting cardiac mitochondrial function and energy production, reducing oxidative stress and protecting endothelial tissues. They also have antiplatelet activity, promote healthy blood lipid levels and healthy blood pressure, improve cell membrane and vascular endothelial function, as well as reducing pro-inflammatory mediator concentrations (eicosanoids, prostaglandins, leukotrienes, and resolvins). [26-29,34]

Notably, this is acknowledged and supported by various government health departments around the world who recommend fish oil consumption in either food or supplemental form for maintaining the health of the cardiovascular system (NHMRC, The Australian Heart Foundation, The American Heart Association, The World Health Organisation etc.), and these actions translate into clinically relevant outcomes. Findings from a broad body of evidence demonstrate a dose-dependent beneficial effect of omega-3 supplementation for improving cardiovascular health as measured by several clinical and biological endpoint subtypes, particularly in those with pre-existing cardiovascular disease.[28-31,34]

Omega-3 PUFAs can reduce endothelial dysfunction and arterial stiffness. Oxidative stress can induce endothelial dysfunction by reducing nitric oxide bioavailability – NO is essential for healthy vasodilation and has an anti-atherosclerotic action.[32] This, along with DHAs ability to fluidise membranes, makes these compounds extremely valuable for optimal endothelial health and function.

Being incorporated into cell membranes also has other significant effects – particularly in myocardial cells. When assimilated into cardiac cell membranes EPA and DHA have the ability to alter the electrophysiological properties of the myocardium which can improve heart rate variability. Low heart rate variability is an independent risk factor for CVD mortality in populations ranging from healthy individuals right through to subjects with congestive heart failure.[34]

It is thought that the positive effect of n-3 PUFAs on blood lipids is due to their activity in the liver and their ability to inhibit the production of very low-density lipoprotein (vLDL),[33] increase fatty acid β-oxidation, and increase production of phospholipids rather than triglycerides by altering enzyme activity.[34]

Nervous System

DHA is highly prevalent in the brain. It is estimated that 20% of the dry weight of the brain is comprised of PUFAs.[19] Once ingested, ω-3 PUFAs enter the cerebrospinal fluid and are transported to the blood brain barrier, where they are attached to brain specific "carrier particles" which can increase intracerebral transport by up 10 times.[3]

Optimal intake of ω-3 PUFAs is necessary for normal brain, structure, function and health at all life stages.[21,22] Specific mechanisms of ω-3 fatty acids that are involved in supporting healthy brain function include being a key cell membrane component thus promoting optimal membrane fluidity, neurogenesis, neuroplasticity, polarity, axon and dendrite maintenance, cell shape, and intracellular signalling. DHA also contributes to synaptic integrity, being involved in optimal synaptic formation, transmission, plasticity, and repair processes. It may also play a role in inhibiting pro-inflammatory mediator-driven neuroinflammation.[8,21,25]

Omega-3 enhances neurotransmitter signalling and binding in the brain by improving membrane fluidity in the lipid bilayer.[19] Furthermore, low DHA intake may impact neurotransmitter production in the brain, including acetylcholine, dopamine, serotonin, glutamate, norepinephrine and gamma-aminobutyric acid (GABA).[19]

Clinical evidence demonstrates a beneficial effect of ω-3 supplementation in young and middle-aged adults and older individuals.[20,21] A comprehensive systematic review found that in young adults, ω-3 supplementation improved endogenous fatty acid profiles, reading abilities (phonologic decoding time and visual analysis time) and also enhanced neurocognitive function.[21] Whilst other research into healthy ageing subjects notes that ω-3 PUFAs may even help to slow cognitive decline.[20] Improvements observed in middle-aged adults included enhanced memory functionality and brain structure (white matter microstructural integrity and grey matter volume), while in older individual's, ω-3 supplements protected against neurodegeneration.[21]

Animal studies have shown that diets rich in ω-3 PUFAs also maintain the integrity of the blood-brain barrier, which is associated with better cognitive function.[20]

Foetal & Infant Development

Maternal DHA levels begin to decline in the third trimester of pregnancy as demands from the rapidly growing foetus increase. Supplementation can help to replenish reduced levels in the mother.[18]

The third trimester marks a period of rapid brain growth in the foetus. This sees a marked increase in DHA accumulation in various tissues including the brain, nerves and the rod photoreceptors of the retina effecting the development of the brain (cognition), eyes (vision) and nerves (neurotransmission).[18,19,23] DHA is also required during this time in the pregnancy to ensure the optimal development of sensory and perceptual systems in the growing foetus.[24] Infants born prematurely can miss out on this period of peak accumulation. This, combined with the fact that DHA accumulation continues for around 10 months post-partum, means that maternal

supplementation with ω-3 fatty acids during the breastfeeding period could be vital, as it helps to maintain DHA levels in the breastmilk which is then passed on to the infant.[19]

DHA in infancy is vital for the developing eye and visual function (particularly for visual acuity), brain and cognitive development, synapse formation, learning and behaviour,[19] as well as immune function.[25]

Table 2: Food sources of EPA and DHA

Eicosapentaenoic Acid[3,16]	Docosahexaenoic Acid[17,18]
Cold water oily fish including salmon, mackerel, herring, sardine, anchovy, tuna and halibut	Cold water oily fish including salmon, mackerel, herring, sardine, anchovy, tuna and halibut
Oysters	Crustacea such as lobster, prawn and crab
Crustacea such as lobster, prawn and crab	Molluscs such as clams and oyster
Seaweed	Octopus
Marine micro-algae	Seaweed
Beef steak and liver	Marine micro-algae
Lamb	Fish liver oils
Caribou	Beef offal
Chicken	Lamb and pork brain
	Chicken
	Eggs (yolk or whole – not in egg whites)

References supplied on request.