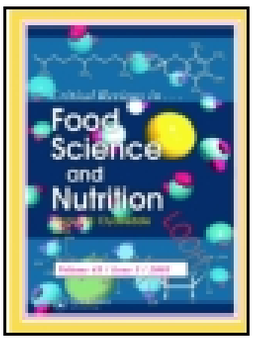

Critical Reviews in Food Science and Nutrition

ISSN: 1040-8398 (Print) 1549-7852 (Online) Journal homepage: http://www.tandfonline.com/loi/bfsn20

Dietary DHA, Bioaccessibility, and Neurobehavioural Development in Children

Carlos Cardoso , Cláudia Afonso & Narcisa M. Bandarra

To cite this article: Carlos Cardoso , Cláudia Afonso & Narcisa M. Bandarra (2017): Dietary DHA, Bioaccessibility, and Neurobehavioural Development in Children, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1338245

To link to this article: http://dx.doi.org/10.1080/10408398.2017.1338245

Dietary DHA, Bioaccessibility, and Neurobehavioural Development in Children

Carlos Cardoso[a,b*], Cláudia Afonso[a,b**], Narcisa M. Bandarra[a,b]

[a] Department of Sea and Marine Resources, Portuguese Institute for the Sea and Atmosphere (IPMA, IP), Rua Alfredo Magalhães Ramalho 1495-006 Lisbon, Portugal

[b] CIIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal

[*]Corresponding author Carlos Cardoso, phone number: (00351) 213027069, e-mail address: carlos.cardoso@ipma.pt

[**]Corresponding author Cláudia Afonso, phone number: (00351) 213027000, e-mail address: cafonso@ipma.pt

Abstract

DHA is a key nutritional n-3 polyunsaturated fatty acid (n-3 PUFA) and needs to be supplied by the human diet. High levels of DHA intake appear to reduce the risk of depression, bipolar disorder, and mood disorders. On the basis of these connections between DHA and neurological health, this paper reviews what is currently known about DHA and children neurodevelopment as well as the benefits of DHA intake to prevention of autism and behaviour disorders through a selective and representative revision of different papers ranging from pure observational studies to randomized controlled trials (RCTs). This review also highlights the issue of DHA bioaccessibility and its implications to the performance of studies. As main conclusions, it can be mentioned that high DHA intake may prevent autism disorder. However, more studies are required to strengthen the connection between autism and dietary DHA. Regarding behavioural disorders, the evidence is also contradictory, thereby raising the need of further studies. From all screened studies on autism, ADHD, and other disorders, it can be concluded that study samples should be larger for greater statistical significance and RCTs should be more carefully designed.

Keywords:

DHA; children; neurobehavioural development; diet; bioaccessibility/bioavailability

1 -- Introduction

The neurobehavioural development of children is modulated and affected by a multi-factorial array of aspects. These aspects comprise nutritional aspects, as shown by an extensive scientific literature on the subject, which hints at a significant correlation between adequate diet and healthy brain development and operation. It is well known that nutrients, such as vitamins and minerals, have a strong effect on DNA synthesis, cell functioning and proliferation, neurotransmitter and hormone metabolism, and the brain enzymatic machinery (Lozoff and Georgieff, 2006; Zeisel, 2009; Zimmermann, 2011; Nyaradi et al., 2013). Moreover, it has been reported that overweight/obese infants between 6 and 24 months of age show lower cognitive scores than their normal-weight peers (Camargos et al., 2016). This suggests a connection between unbalanced diets and suboptimal neurological development. Taking into account that the most rapid brain changes occur during the first 2 years of life (Johnson, 2001), this period is critical for neurodevelopment (Taanila et al., 2005). The brain is subjected to rapid evolution and minor disturbances in this process and associated phenomena may have long-term effects on the neuronal structures and functional ability (Grantham-McGregor et al., 2007). Therefore, assessing the impact of a mother's diet during pregnancy and breastfeeding and of an infant's diet in the first decisive years is crucial for understanding different outcomes in the behaviour, intelligence, and broad neurological development of the children and, obviously, of the future adults.

There are different nutrients in food that have an effect on brain health, such as geniposide, apocynin, eicosapentaenoic (20:5 n-3, EPA) and docosahexaenoic acid (22:6 n-3, DHA) (Editorial, 2015). In particular, DHA, one of the most important marine n-3 polyunsaturated fatty acids (n-3 PUFAs), may have a strong influence on brain health (Freemantle et al., 2006). Indeed, consumption of larger amounts of n-3 PUFA, particularly DHA, appears to reduce the risk of depression (Hibbeln and Davis, 2009), including postpartum depression, bipolar disorder (manic depression), schizophrenia, and mood and behaviour disorders (Milte et al., 2012). It has also been hypothesized a connection between DHA in the diet and in the nerve cell membrane and the risk of dysfunction of the central nervous system in the form of anxiety (Ross, 2009), irritability, susceptibility to stress (Hellhammer et al., 2012), dyslexia (Stordy, 1999), stereotypic

behaviour, aggressiveness (Gow and Hibbeln, 2014), reduced learning capacity (Milte et al., 2012), impaired memory and cognitive functions, and extended reaction times (Stonehouse et al., 2013).

Regarding neurobehavioural development, it is worth mentioning that DHA is rapidly incorporated in the nervous tissue of retina and brain during the brain's growth spurt, which occurs from the last three months of pregnancy up to two years of age (Martinez, 1992). Positive findings on a beneficial effect of DHA supplementation or fish intake during pregnancy and/or lactation on developmental outcomes of the offspring have been reported from both observational studies and randomized controlled trials (RCTs) (Osendarp, 2011). However, whereas evidence from term infants shows that visual acuity is improved by DHA (and other long chain n-3 PUFAs), for cognitive function, improvements remain controversial (Osendarp, 2011). Another area of effects is children behaviour and associated disorders.

Accordingly, this review paper will be directed to the role of DHA in the development of the nervous system and behavior aspects as well as in the prevention and mitigation of autism. The state-of-the-art in these scientific areas of research will be analysed taking into account the DHA chemical form, that is, the wider chemical structure where DHA is bound (triacylglycerol, TAG, free fatty acid, FFA, ethyl ester, EE, and, phospholipid, PL) and its effects on DHA bioaccessibility and bioavailability.

2 -- DHA Importance in the Central Nervous System

The relationship between DHA and neurobehavioural aspects is part of a larger picture where DHA has several ways of affecting the whole nervous system. Both neural integrity and function can be permanently disturbed by deficits of n-3 PUFA during foetal and neonatal development (Anderson, 1994). DHA, as other n-3 PUFA, has an important role in the nervous system, particularly in the central nervous system (CNS), which is highlighted by its prominence in neural tissues' composition (Anderson and Connor, 1994; Youdim et al., 2000). Indeed, DHA is by far the main n-3 PUFA present in the brain ---its content within brain fatty acids is 12-15% (Whelan, 2008)---, where it is predominantly located in neuronal membranes of the gray matter, especially in synapses (Cunnane et al., 2013). In the CNS, DHA is mainly found in PL, being the

unesterified DHA undetectable under basal conditions (Sun et al., 2004). In addition, the brain fatty acid-binding protein preferentially binds DHA (and other n-3 PUFA) (Hanhoff et al., 2002), leading to higher levels of DHA incorporation in the molecular structures of the membranes (Storch and Thumser, 2010).

It should be remarked that DHA in the CNS is supplied by the liver. In this organ, □-linolenic acid (18:3 n-3, ALA) is elongated and desaturated into EPA and then to tetracosahexaenoic acid (24:6 n-3), which is converted by peroxisomal □-oxidation to DHA (Voss et al., 1991). Moreover, in the liver, DHA from food is taken up and sent to other parts of the organism (Scott and Bazan, 1989). For reaching the brain, DHA transport by simple diffusion through the blood brain barrier, BBB (Hamilton and Brunaldi, 2007), is a possibility. But, there is also evidence suggesting the expression and functional role of fatty acid transporters at the BBB (Mitchell and Hatch, 2011). An alternative to attain DHA from food is its conversion from ALA, a precursor of DHA. However, the level of ALA in the diet does not correlate with the level of DHA, thus justifying supplementation of the nursing mother's diet with DHA (Ratnayake and Hollywood, 1997). Indeed, typically in humans, despite the availability of the required enzymes, DHA synthesis is minimal (Plourde and Cunnane, 2007). Even with a diet poor in DHA, brain cells have limitations concerning DHA synthesis from ALA (Igarashi et al., 2007). Furthermore, it should be remarked that plasma or red blood cell DHA does not correlate well with DHA in the brain cells (Cunnane et al., 2013).

The PLs of the synaptic plasma membrane and synaptic vesicles are rich in DHA (Glomset, 2006). Throughout childhood development DHA is accumulated within the brain PL, PC and PE (Martínez and Mougan, 1998). Concerning this issue, it has been reported that continuous supply of DHA, but not arachidonic acid (AA, 20:4 n6), may augment brain phosphatide and synaptic protein levels in accordance to animal models (Cansev and Wurtman, 2007). Phosphatidylserine (PS) is also of great importance and found in large amounts in the human brain, containing non-negligible levels of DHA (Guo et al., 2007).

On the other hand, DHA is not a source for eicosanoid synthesis, thereby differing from EPA. DHA exerts its influence directly and indirectly. However, DHA can also be converted to EPA

by a retroconversion route, thus generating different eicosanoid metabolites (Conquer and Holub, 1997). Nonetheless, DHA derivatives produced by oxidation are also important, being named docosanoids (Sawazaki et al., 1994). These docosanoids are not very different from eicosanoids and are considered potential mediators of relevant biochemical mechanisms in the CNS (Sawazaki et al., 1994). Docosanoids encompass NPD1, neuroprostanes (NeuroPs), maresins, and other compounds (Bazan et al., 2011). In particular, NeuroPs are related to prostaglandins and form an important group of oxidized cyclopentanoid derivatives. NeuroPs are attained through a multi-phase non-enzymatic radical mechanism from the non-enzymatic DHA peroxidation in neuronal cells (Porta et al., 2013). But, it was found that lipoxygenase inhibitors are detrimental to the synthesis of various docosanoids (Bazan et al., 1984).

Specifically, NPD1 is formed by selective DHA oxygenation catalysed by 15-lipoxygenase-1 (Calandria et al., 2009). In response to cellular and systemic disturbances, NPD1 favours homeostatic signalling (Lukiw et al., 2005). The positive regulatory activity of NPD1 combined with DHA follows different mechanisms (Brand et al., 2008; Lukiw and Bazan, 2008; Rapoport et al., 2007).

Moreover, the efficiency of various membrane transporters and enzymes is affected by DHA incorporation into cell membranes (Srinivasarao et al., 1997). This is of great importance in that many critically relevant cellular phenomena take place in and on membranes (Klose et al., 2013). These phenomena are influenced by the biochemical as well as biophysical properties of organelle membranes. In fact, the membrane lipid composition affects its properties, namely exerting an effect upon the action of membrane-embedded proteins (Lee, 2011).

Physical properties of membranes are influenced by DHA. Indeed, the physical properties of membranes are affected both by the head groups and the hydrocarbon chains of lipid molecules. These aspects can be influential not only to the properties, but also to the biochemical phenomena in the membranes, even with small changes in lipid composition (Klose et al., 2013). Namely, whereas a hypothetical bilayer of PC with two chains of a saturated fatty acid such as palmitic acid (16:0) shows a packed ordered state, a bilayer of PC with two DHA chains displays a less ordered state with less constrained lipid molecules (Kahya et al., 2003; Silvius, 1982).

Besides, larger fatty acid chains and a higher percentage of SL and sterols in the membrane are associated with thicker membranes (Surma et al., 2011). Therefore, the connections between DHA and the membrane physical properties are another important research field deserving further scientific studies.

3 -- DHA and the Neurological Development

A deeper understanding of the functional and structural development of the neurological system has emerged from a varied array of techniques (encompassing clinical lesion and experimental animal studies) and, more recently, as a result of greatly ameliorated neuroimaging methodologies, particularly Magnetic Resonance Imaging (MRI) and Positron Emission Tomography (PET) (Levitt, 2003; Uddin et al., 2010).

The effects of DHA on neurological development need an understanding of the multiple connections between DHA and the highest degrees of brain activity. A deficient level of DHA is related with changes in the operation of the neurological system (Bazan et al., 2011). Conversely, higher dietary intake of DHA is linked to better brain health (Burri, 2015). Indeed, DHA is enriched in synaptic membranes, being able to change their fluidity as well as neurotransmitter and receptor densities. These mechanisms whereby DHA affects neural cells were already described in a previous section, but more studies on their details and the way that DHA positively affects neurological operation are warranted. Namely, there are several studies of a medical nature pointing to the positive effect of DHA on cognitive function (Burri, 2015), but the full understanding of the underlying biochemistry remains elusive.

Many studies relate to human neurological development during pregnancy and in infancy. Particularly, studies have shown that maternal DHA dietary intake during the last trimester of pregnancy improves cord blood DHA status (Guesnet and Alessandri, 2011). Indeed, during pregnancy, DHA is transferred across the placenta to the fetus in high quantities (Haggarty et al., 1997), where it is stored in developing neural tissues, particularly during the fetal brain growth spurt in the last pregnancy trimester (Martinez, 1992). The frontal regions of the brain are a primary area of DHA accumulation and experience rapid growth during this period. Such brain regions, namely, the frontal cortex, are crucial for language, memory, and higher order cognitive

operation, including purposeful behaviours (Anderson et al., 2008). The fundamental role of appropriate DHA supply during this crucial period of brain development is shown in studies of preterm infants who do not experience full gestation period and concomitant DHA accumulation. These preterm infants display lower concentrations of DHA in brain tissues (Haggarty et al., 1997) and show higher risk of developmental delay (Anderson and Doyle, 2003), impaired executive functioning (Anderson and Doyle, 2004), and attention deficit/hyperactivity disorder, ADHD (Bhutta et al., 2002), when subjected to comparison with term-born infants.

Furthermore, regardless of gestation period, it has been reported that maternal intake of DHA benefits infant sleep patterning, which may be regarded as an early neurodevelopmental measure (Judge et al., 2012). Other benefits ranging from attention (Colombo et al., 2004) to problem-solving (Judge et al., 2007a) and visual acuity (Judge et al., 2007b) have been claimed. Another study showed that preterm infants fed DHA-enriched formula had a higher Mental Development Index score at 12 months than controls fed the typical preterm formula (Carlson et al., 1994). Developmental benefits related to DHA enrichment in maternal diet seem to extend into early childhood with better intelligence quotient reported for four-year-old children with 1,183 mg of daily DHA (Helland et al., 2003) and enhanced neurological development at 5.5 years of age using a lower dose of 500 mg DHA every day (Escolano-Margarit et al., 2011). However, another study relying on mothers receiving DHA supplements did not find any significant difference in general cognitive functioning of children at 18 months and four years (Gould et al., 2016).

It can be argued that supplementation needs to be kept after birth during the first critical years of child's brain development. Particularly, the first six months after birth are decisive and it has been reported that the brain experiences the highest accumulation of DHA during this period: brain DHA accretion is about half the whole body DHA accretion, thereby suggesting a very selective DHA uptake across the BBB during this period (Bernardi et al., 2012). Regarding the time after birth, an important study involving four-year-old children showed that those fed a DHA-enriched formula displayed full-scale and verbal IQ scores (Wechsler Pre-School and Primary Scale of Intelligence, WPPSI) higher than the others in the control group (Gale et al., 2010). On the other hand, IQ scores (WPPSI-Revised) of six-year-old children who were fed a

formula containing long chain PUFA (including 210 mg DHA per 100 g) did not differ from scores of children in the control group (Willatts et al., 2013). The use of a mixture of PUFA instead of only DHA may be a factor explaining the opposite conclusions of these studies.

There have been other studies, including RCTs (McCann and Ames, 2005). These authors have reviewed these and other types of studies and have concluded that particularly studies in animals suggest that, within the context of specific experimental designs, changes in brain concentrations of DHA are positively associated with changes in neurological development.

Of course, there are various factors that affect the quality of the studies' results. Many studies were not clear about the used processes to independently generate the randomisation sequence or just used inadequate processes for this purpose (Gould et al., 2013). There are also potential biases such as systematic post-randomisation losses, being frequently observed a greater attrition in the DHA-supplemented group with respect to the control group (Helland et al., 2003; Helland et al., 2008). Moreover, high attrition (ranging from 27% to 86%) has been observed in these studies, which compounds to the fact that many trials were relatively small and, as such, unable to detect statistically significant differences in neurological development (Campoy et al., 2010; Colombo et al., 2004; Decsi et al., 2005; Dunstan et al., 2008; Helland et al., 2003; Helland et al., 2008; Judge et al., 2007a; Kannass et al., 2009; Meldrum et al., 2015; Smuts et al., 2003; Tofail et al., 2006; van Goor et al., 2011).

4 -- The Role of dietary DHA in Autism

Autism is an important pervasive developmental disorder (PDD), whose occurrence frequency seems to be increasing in the recent decades (Ratajczak, 2011). It is characterized by impaired communication and social interaction and repetitive behaviours. Researchers point to various genetic, environmental, and immunological aspects as causes of autism (Kidd, 2002). It is also known that low birth weight and pre-term birth double the risk of autism (Schendel and Karapurkar, 2008). In particular, one line of evidence links diet composition to autism (Dufault et al., 2009). Within the various nutrients, low PUFA levels have been associated to autism (Amminger et al., 2007; Meiri et al., 2009; Yui et al., 2012).

Some authors have linked the observed rise in the prevalence of autism disorders to the increase in the n-6 PUFA/n-3 PUFA ratio in the human diet (van Elst et al., 2014). Canola (cultivar of rapeseed) and soybean oils ---rich in n-6 PUFA--- production has greatly expanded in the 80's and 90's decades of the past century and their consumption experienced a dramatic increase, changing n-6/n-3 PUFA in human diet not only directly, but also indirectly via incorporation in livestock diet (Blasbalg et al., 2011). This has led to n-6/n-3 PUFA ratios of 10-25:1, very high values with possible implications to human health (Cordain et al., 2005). In particular, it has been argued that a change in the ratio of n-6/n-3 PUFA, especially during early life, may lead to developmental changes in brain connectivity, synaptogenesis, cognition, and behaviour that are associated to a higher incidence of autism disorders. Moreover, there is some evidence showing that incorporation of n-3 PUFA in a diet may decrease symptoms in children with autism (Meguid et al., 2008). This connection between n-3 PUFA and autism is also supported by the association between autism and mutations in genes encoding FA binding proteins expressed in the brain (Maekawa et al., 2010). It has also been noted that sex difference in metabolic conversion of PUFAs agrees with the higher frequency of autism disorders in males (Burdge and Wootton, 2002). Indeed, these authors have argued that women may possess a greater capacity for ALA conversion to DHA than men, due to the importance of meeting the DHA requirements of the fetus and baby during pregnancy and lactation.

Regarding the mechanistic aspects underlying an n-3 PUFA-autism disorder connection, the significant effects of n-3 PUFA on neuronal morphology, such as synapse formation, have been reported as very important (Delorme et al., 2013). It has also been pointed out that autism disorders are associated to the disruption of white matter tracts producing brain connectivity alterations (Won et al., 2013) and white matter tract formation is very sensitive to the n-6/n-3 PUFA ratio (Durand et al., 2013).

A possible mechanism relating DHA deficiency in the diet to autism may involve brain-derived neurotrophic factor (BDNF), a protein active in brain areas very important for learning, memory, and higher thinking (Yamada and Nabeshima, 2003). In fact, it has been claimed that the serum levels of BDNF in human patients with autism are significantly lower than those of the control group (Hashimoto et al., 2006). On the other hand, it is known that DHA protects neurons and

glia from death, among other mechanisms, by supporting high levels of BDNF (Lukiw and Bazan, 2008).

Another route for the effects of DHA on autism disorders may be associated to neurotransmitter physiology, for instance, production of dopamine and serotonin. A reduction in DHA in rodent brains has been correlated with changes in levels and signalling of neurotransmitters (Müller et al., 2015). DHA deficiency in the diet led to lower serotonin levels in the frontal cortex of tested animals (de La Presa Owens and Innis, 1999). However, this study did not separate the effects associated to DHA deficiency from those due to insufficient AA. The importance of low concentrations of neurotransmitters in the etiology of autism is based on studies that, for instance, show lower levels of serotonin in autistic children (Chugani et al., 1999; Pardo and Eberhart, 2007). Moreover, there are studies that point to an effect of DHA deficiency on the activity of □-aminobutyric acid (GABA)-ergic receptors (Takeuchi et al., 2003), which may be an important way of DHA helping in the prevention of the pathogenesis of autism disorders (Hamazaki and Hamazaki, 2008; Pardo and Eberhart, 2007), since a loss of activity of these receptors has been observed in the brain tissue of autistic individuals.

A defective FA □-oxidation has been reported for autism, being proposed that autism is a disorder of mitochondrial dysfunction (Lombard, 1998). More specifically, autism disorders have been associated with a disorder of FA metabolism through a possible dysfunction of mitochondrial □-oxidation by long chain acyl-CoA dehydrogenase (Clark-Taylor and Clark-Taylor, 2004). This may lead to reduced synthesis of DHA.

The linkage between autism and inflammatory processes is also worth investigating. Some rodent models of prenatal infection have offered insights into the possible role of neuroinflammation in autism disorders (Schwartzer et al., 2013). These models have generated data suggesting that maternal inflammation alters aspects of rodent neuronal function and behaviour in a way consistent with dysfunctions observed in autism (Malkova et al., 2012). On the other hand, it has been shown that diets rich in DHA lead to a reduction in neuroinflammatory responses to a wide variety of substances (Orr et al., 2013). Accordingly, it has been observed that dietary DHA protected mice offspring from the deleterious effects of

gestational exposure to a viral mimetic substance on autism behaviour parameters (Weiser et al., 2016). These authors concluded that elevated dietary levels of DHA, especially during pregnancy and nursing, may help protect normal neurodevelopment from the possible adverse consequences of maternal infection and, at least, alleviate autism symptoms in the offspring.

The recent interest in the gut:brain axis has offered an alternative path for the DHA action against autism. Evidence points to the essential role of gut microbiota composition and metabolism in brain development and disease risk throughout life (Tuohy et al., 2015). Gut microbiota is a key player in development and regulation of the gut:brain axis and might thus play a critical role in autism pathophysiology. Indeed, gut microbiota is different in autistic individuals (Tuohy et al., 2015). The gut microbiota may affect the metabolism of many different molecules, namely, unsaturated fatty acids such as DHA, and may also be affected by DHA levels in the diet.

These possible mechanisms are still not fully understood and the evidence ascribing anti-autistic effects to high DHA levels in the diet is still not conclusive and convincing (Table 1). Namely, there are studies using rats in autism models, for instance with valproic acid-induced rat autism (Gao et al., 2016), which raise doubts regarding the applicability of their conclusions to humans. These authors claimed that DHA may play a neuroprotective role in hippocampal neuronal cell and ameliorate dysfunctions in learning and memory in the used animal models and concluded that DHA could be used as treatment against behavioural dysfunctions in autism. This means extrapolating too much from animal data. Furthermore, the used dosage, 300 mg/(kg.day) DHA, was extremely high.

Intervention studies using human subjects are difficult, thus entailing more importance of observational studies. An interesting recent study (Jory, 2016) showed that the red blood cell and serum DHA levels may be lower in children with autism in comparison with control children. However, the population sample was very small, underlining a need for larger age- and sex-matched investigations in the group of autistic children. Another study with a larger sample (40 autistic children *versus* 40 control children, all under 5 years old) showed that autistic children

had lower dietary consumption of foods containing DHA as well as lower levels of serum DHA than children in the control group (Al-Farsi et al., 2013).

There have been other studies reporting decreased levels of DHA and, usually, with normal levels of its n-3 PUFA precursor ALA and normal n-6 series PUFA (Vancassel et al., 2001). Low plasma DHA levels were found in more than two thirds of the autistic children in a study with 80 autistic children and 80 healthy-matched children, aged between 4 and 12 years (Mostafa et al., 2015). These authors also found an association between low DHA plasma content and elevated serum levels of brain-specific auto-antibodies. Another study with 30 autistic (and 30 healthy) children found that DHA levels were significantly lower in the autistic children compared with the control group (Meguid et al., 2008). Moreover, the mean ratio of AA/DHA was significantly higher in autistic children compared with the control group. After this assessment, there was an intervention study over a trimester that involved a DHA supplementation of 240 mg and vitamin E in the clinical group (Meguid et al., 2008). The majority of the autistic children (two thirds) showed clinical and biochemical improvement, being elevated DHA levels in the supplemented group measured by blood analysis. A double-blind, randomized, placebo controlled trial (Yui et al., 2012) reported significant improvement in Social Responsiveness Scale communication subscale scores after 16-week of 240 mg/day AA and 240 mg/day DHA treatment (n = 7), as compared to placebo treated (olive oil, n = 6) autistic patients. Unfortunately, the significance of this study is reduced by the small number of participants.

However, it has also been claimed no differences in DHA content in the red blood cell polar lipids of children diagnosed with autism in comparison to control (Bell et al., 2004). Other studies do not find strong evidence for an abnormal FA metabolism in the pathogenesis of autism disorders in general (Bu et al., 2006). Regarding intervention trials, there are also some studies reporting no effect. For instance, a double-blind randomized trial of 48 children on a dietary DHA supplementation of 200 mg/day for 6 months reported no improvement in the core symptoms of autism (Voigt et al., 2014). Likewise, a six-month, randomized, placebo controlled trial in autistic children 2 to 5 years of age with 2 groups of 19 patients each showed no significant differences between groups, thereby not supporting a high DHA dosage in the diet

(Mankad et al., 2015). However, this study was not a pure DHA test, since it involved EPA supplementation (a total of 1.5 g EPA+DHA). It may also be argued that dietary DHA only makes a difference if administered to pregnant and nursing mothers or in infants' milk formulas.

Other studies have tried a different approach with a more detailed analysis of the FA profile and DHA levels through the determination of DHA concentration in specific lipid classes and groups. Unlike EPA, which is oxidised once enters the brain from the plasma, unesterified DHA is largely (>80%) and selectively delivered via an acyl-CoA synthetase and acyltransferase to the sn-2 position of membrane phospholipids ---the main lipid class in cell membranes, -, particularly phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (Rapoport, 2013). In one important study, plasma lipids were fractionated in cholesterol ester (CE), PC, lysophosphatidylcholine (LYPC), PE, TAG, diacylglycerols (DAG), and FFA and their DHA content was determined in a large case-control study, 153 children with autism and 97 developmentally normal controls, known as Childhood Autism Risk from Genetics and the Environment, CHARGE (Wiest et al., 2009). These authors reported that DHA content in PE was lower in children (2-5 year old) with autism than in the control group. In macrophages, it was also found that the DHA content in PE is directly affected by the ability to synthesize plasmalogens (Gaposchkin and Zoeller, 1999), thereby pointing to the importance of the peroxisome function, since DHA and plasmalogens have synthetic pathways with steps occurring in the peroxisome (Farooqui and Horrocks, 2001).

Autism can be related to other health problems affecting the brain and nervous system. In fact, many studies have outlined the dimensionality of autism concerning its co-morbidity with other neurodevelopmental disorders such as ADHD, dyslexia, dyspraxia (Richardson and Ross, 2000). This makes understanding the etiology of autism disorders a central issue in children neurodevelopmental studies. In regard to this, more studies are required to strengthen the connection between autism and the n-3 PUFA, with particular emphasis on the DHA, thereby separating DHA effects from those of other PUFAs and identifying dietary DHA-autism causality links or mere DHA-autism associations pointing to other causes. A more detailed analysis involving the determination of the DHA content in each lipid group (TAG, PE, PC, etc.) seems to be of paramount relevance. It is also very important to have larger groups for greater

statistical significance and to carefully design RCTs (crossover or not) that allow to draw clearer conclusions. Moreover, the gut-brain axis needs to be regarded as a crucial issue in all studies and be accounted for. Finally, DHA bioaccessibility and bioavailability of the supplements must be taken into account in selecting effective DHA dosages for the RCTs.

5 -- DHA and Behaviour Disorders

There are several important behaviour disorders affecting children ranging from emotional dysregulation/lability (EL) to oppositional behaviour and developmental coordination disorder (DCD), with ADHD one of the best studied and more frequently correlated with dietary components. Of course, there are overlapping zones between different behaviour disorders (Cooper et al., 2016). ADHD is characterized by insufficient attention, excessive activity, or difficulty controlling behaviour that is not adequate for a person's age and it is considered a neurodevelopmental disorder (Bradshaw, 2001).

There have been studies supporting a relationship between diet, namely n-3 PUFA, and several behavior disorders (Bloch and Qawasmi, 2011; Richardson et al., 2012; Sonuga-Barke et al., 2013) (Table 1). The connection between essential fatty acids and ADHD in children has been the subject of much research. Deficiency of long chain PUFAs has been associated with ADHD (Janssen and Kiliaan, 2014). In this context, it was reported that 53 children with ADHD had significantly lower plasma levels of n-3 PUFA than did the control group of 43 children (Stevens et al., 1995). However, studies are sometimes contradictory and leave many unanswered questions. The quality of the experimental design, the degree of detail in data collection and their analysis, and the ability to exclude confounding variables are essential for the achievement of reliable results. For instance, whereas, in the primary analyses, n-3 PUFA supplementation did not show improvements in measures of EL, oppositional behaviour, conduct problems or aggression, subgroup analyses in studies with strict inclusion criteria found a significant reduction in EL and oppositional behavior (Cooper et al., 2016). However, results excluded the possibility of moderate to large effects. Moreover, studies should clearly circumscribe the behaviour disorders to be targeted by them and distinguish between DHA and other n-3 PUFA, given their different properties. In this case, a recent study showed that DHA supplementation

(600 mg/day from algal oil) appears to offer a safe and effective way to improve reading and behaviour in healthy but underperforming children (Richardson et al., 2012). Besides observational or RCTs, experimental works investigating the mechanistic aspects that relate dietary DHA to behaviour disorders during the neurodevelopment of children are crucial.

With respect to these mechanistic aspects, there has been some progress, with particular emphasis in the area of ADHD (Schuchardt et al., 2010; Zimmer et al., 2002). An important mechanism relating DHA to ADHD may be dependent on neurotransmitter physiology, namely dopamine transmission. Indeed, standard pharmacological treatment for ADHD involves medications that increase dopamine availability. It is therefore noteworthy that chronic n-3 PUFA deficiencies can reduce dopamine and its binding to D2 receptors both in frontal cortex and other brain regions, and are related to attentional and behavioural dysfunctions similar to those observed in ADHD (Zimmer et al., 2002). Dysregulated phospholipid metabolism has also been proposed as an underlying biological component of neurodevelopmental disorders such as ADHD (Brown and Austin, 2011). The brain phospholipid metabolism may be altered because of an increased rate of loss of EPA, AA, and DHA from the sn-2 position of phospholipids. This causes changes in the operation of the membrane-associated proteins and of the cell signaling systems.

An overview of the studies linking dietary DHA and ADHD shows only limited support for the efficacy of DHA supplementation for the core symptoms of ADHD. Throughout the last decades, studies have sometimes reported DHA influence upon or association to ADHD. A study that compared 44 hyperactive children with 45 control children (age- and sex-matched) found that DHA levels were significantly lower in the hyperactive children, who also had auditory, visual, language, reading, and learning difficulties, and lower average birth weight compared to controls (Mitchell et al., 1987). In another study (Colter et al., 2008), it was also reported that ADHD children had lower levels of DHA and total n-3 PUFA than the control group. A prospective, longitudinal study in Arctic Quebec involving 154 children provided neurophysiologic and neurobehavioural evidence of long-term beneficial effects of DHA intake *in utero* (umbilical cord DHA) upon performance in neurobehavioural assessments of memory (Boucher at al., 2011).

There are also intervention studies. A very recent randomized controlled three-way crossover trial (Milte et al., 2015) has shown that increasing erythrocyte DHA and EPA contents via dietary supplementation may improve behavior, attention, and literacy in children with ADHD, with particular importance to a supplement very rich in DHA (approximately an EPA:DHA ratio of 1:4). This study involved 90 children (53 completed treatment) that were randomized to consume supplements high in EPA, DHA, or linoleic acid (control) for four months each in a crossover design. Erythrocyte FAs, attention, cognition, literacy, and Conners' Parent Rating Scales (CPRS) were measured. In children with blood samples, increased erythrocyte EPA + DHA (from 4.09 ± 0.85 to 8.78 ± 1.36% of total FAs) was associated with improved spelling, attention, and reduced oppositional behavior, hyperactivity, and cognitive problems (Milte et al., 2015). On the other hand, a double-blind placebo RCT with 95 children diagnosed with ADHD found improved working memory function, but no effect on other cognitive measures and parent- and teacher-rated behaviour in the study population (Widenhorn-Müller et al., 2014). A low DHA daily dose supplementation (120 mg/day) is a shortcoming of this study. For other behavioural problems, another RCT comprising 166 children (9-12 year old) reported that after three months of supplementation, DHA-rich fish oil-fortified foods protected against increases in aggression and reduced impulsivity among females (Itomura et al., 2005). However, no significant changes in assessment of aggression or impulsivity were observed in males.

Other RCTs with DHA supplementation against placebo have been less positive. For instance, an RCT involving 63 children (between 6 and 12-year-old) with ADHD reported that a 4-month period of DHA supplementation (345 mg/day) did not reduce ADHD symptoms (Voigt et al., 2001). Moreover, DHA had a low effectiveness against ADHD in another study using an even higher dose of DHA (3.6 g per week or 514 mg/day), since this supplementation did not lead to better outcomes than a mere placebo (Hirayama et al., 2004). Nonetheless, these authors concluded that treatment of ADHD with FAs deserves further investigation ---some differences in particular cognitive aspects were detected---, but careful attention should be paid as to which FA(s) is/are used. For other behaviour issues, a study encompassing 233 children aged 9--14 years and analysing aggression and impulsivity using the Hostility-Aggression Questionnaire for Children (HAQ-C) and the Barratt Impulsiveness Scale, version 11 (BIS-11) found that while

school attendance was higher in the DHA group, no behavioural benefit as a result of DHA supplementation (650 mg/day) was detected (Hamazaki et al., 2008).

As with autism studies, a number of factors seems to fail to reach statistical significance due to inappropriate DHA dosages, small sample sizes, and lack of study homogeneity in terms of design and study participants. Therefore, future studies should encompass larger sample sizes, involve a more thoughtful design ---with inclusion of bioaccessibility and bioavailability factors (see below)---, and a previous objective assessment of the questions to be answered.

6 -- Dietary Sources of DHA

In order to address the shortcomings of some of the previously mentioned studies, a better knowledge of the dietary sources of DHA is very important. Diets should be formulated in order to ensure an adequate level of DHA supply. The main source of DHA is seafood, particularly marine fish and shellfish (Ackman, 1988). DHA is found in the flesh of both lean and oily fish (e.g., herring, salmon, sardine), with much greater amounts in the latter, and in the liver of some lean fish species, such as cod. DHA is present primarily as TAG and, to a lesser extent, as FFA in fish and derived unrefined raw oils (Schuchardt and Hahn, 2013). In krill oil, a third fraction is found, since a substantial percentage of n-3 PUFA (and DHA) is bound in PL (Schuchardt and Hahn, 2013).

Three main classes of fish products may be differentiated on the basis of DHA content: relatively poor DHA sources (black scabbardfish, catfish, hake, megrim, tilapia); moderately rich DHA sources (halibut, pollock); and very rich DHA sources (herring, mackerel, salmon, sardine), corresponding to the approximate ranges <300, 300-500, and >500 mg/100 g, respectively (Afonso et al., 2013a,b; Bandarra et al., 1997; Costa et al., 2015; Harris et al., 2007; Nunes et al., 2006).

For attaining more detailed data concerning DHA concentration in different marine sources see Table 2. It is based on IPMA's extensive database (Bandarra et al., 2004; Nunes et al., 2006) and different papers (Makri et al., 2011; Patil et al., 2007; Peinado et al., 2014; Taoka et al., 2011). The six highest DHA contents are found in the European eel, chub mackerel, Atlantic salmon,

Atlantic mackerel, gilthead seabream (wild), and sardine, all exceeding 1000 mg/100 g (Bandarra et al., 2004; Nunes et al., 2006).

As a result of the data mentioned above and given the importance of DHA for a healthy neurological development of children, it is advisable that pregnant and nursing women eat various fish, including fat fish species, during their pregnancy and lactation period. Unfortunately, studies' conclusions do not allow the establishment of a recommended DHA daily intake aiming at children with adequate neurobehavioural development. Nonetheless, despite the problems with the available evidence, there is a plausible risk to a healthy neurological development as a result of deficient dietary DHA. As a reference, the recommendations issued by the American Academy of Pediatrics for nursing women can be endorsed (Johnston et al., 2012): The mother's diet should include an average daily intake of 200 to 300 mg of the ω-3 long-chain PUFAs (DHA) to guarantee a sufficient concentration of preformed DHA in the milk. Moreover, consumption of one to two portions of fish (e.g., herring, canned light tuna, salmon) per week will meet this need. The concern regarding the possible risk from intake of excessive mercury or other contaminants is offset by the neurobehavioural benefits of an adequate DHA intake and can be minimized by avoiding the intake of predatory fish (e.g., pike, marlin, tile fish, swordfish) (Johnston et al., 2012). Furthermore, poorly nourished mothers or those on selective vegan diets may require a supplement of DHA as well as multivitamins.

7 - DHA Bioaccessibility and Bioavailability

For a better assessment of the DHA supplements' dosages and the impact of dietary options, it is fundamental that instead of dealing with the total contents of DHA in food, particularly seafood and supplements, DHA contents available to intestinal absorption after digestion of a cooked food eaten in a typical meal or after ingestion of a supplement be accounted for using appropriate methodologies. Indeed, the level of DHA in a portion of food that is eaten may be quite different from the bioaccessible level, that is, the DHA concentration that is released from the food matrix into the intestinal lumen after digestion and is available for absorption (Cardoso et al., 2015; Paustenbach, 2000). On the other hand, bioavailability is usually defined as the fraction of an

oral dose of a substance that reaches the systemic circulation (Schumann et al., 1997). The bioaccessible content is always equal to or higher than the bioavailable content (Cardoso et al., 2015). Bioaccessibility can be determined by *in vitro* simulations of human digestion (Cardoso et al., 2015; Versantvoort et al., 2005). For bioavailability, according to the definition given above, *in vivo* and *in vitro* experiments are possible. For the latter, cell lines and transwell assays are used for mimicking the intestinal lining barrier (Minoo et al., 2007) and cell cultures simulating the relevant liver tissues may also be used (LeCluyse et al., 2012).

Hence, a first step towards a more accurate determination of the DHA effectively provided by any given diet entails cooking foods according to the usual culinary methods (Afonso et al., 2015; Costa et al., 2015). Boiling, grilling, roasting, frying and other culinary treatments can alter DHA content either by leaching it out or decomposing it or concentrating it due to water loss. Moreover, cooking generates several biochemical and physical transformations that bring about relevant matrix changes, which, in turn, affect DHA bioaccessibility (Afonso et al., 2015; Costa et al., 2015, 2016) (Figure 1). Indeed, it was observed that grilled fish displayed lower DHA bioaccessibility than raw fish. For instance, the bioaccessibility of DHA in meagre (*Argyrosomus regius*) was reduced from 41% in the raw fish to 33% in the grilled fish (Afonso et al., 2015). A similar DHA bioaccessibility reduction due to grilling was also reported for gilthead seabream (*Sparus aurata*) (Costa et al, 2016).

This was ascribed to a lower lipid bioaccessibility, which may be due to the very harsh thermal treatment (180°C and direct conductive heat) associated to grilling. Under these extreme heating conditions, protein denaturation is enhanced and digestibility is reduced because covalent bonds between polypeptide chains are formed (Dadorama, 1996). In fact, there was also a reduction of protein digestibility with grilling. Therefore, it seems possible that protein aggregates formed during grilling trap a significant portion of the lipids, including DHA, thus reducing DHA bioaccessibility (Afonso et al., 2015).

Besides cooking-related changes, other factors affect DHA bioaccessibility in foods (and supplements), deviating it from the ideal 100%. Indeed, it has been shown that fatty acid bioaccessibility in salmon (*Salmo salar*) is reduced as the number of double bonds increases

(Costa et al., 2015). Hence, DHA with six double bonds (a very high level of unsaturation) is in the lower end of FA bioaccessibility. Whereas erucic acid (22 carbons just as DHA, but one double bond) exhibited a bioaccessibility of 93.9% in raw fish, DHA's bioaccessibility was only 73.5% also in the same raw fish (Costa et al., 2015). This phenomenon may be explained by three main causes: chemical affinity of DHA; digestive lipases favouring the hydrolysis of less unsaturated fatty acids (Akanbi et al., 2014); and location of DHA in the sn-2 position of TAG, which is less accessible to lipases, particularly the important pancreatic lipase (Schuchardt and Hahn, 2013). The two latter causes may lead to lower bioaccessibility because monoacylglycerols (MAG) and DAG may be less easily bioaccessible than FFA. A recent study found evidence supporting a predominantly regioselective action of lipase, which may be meaningful for DHA bioaccessibility (Costa et al., 2016).

Bioaccessibility and, as a consequence, bioavailability of DHA may also depend on the chemical binding form (DHA bound in EE, TAG or PL), matrix effects (fat and other components in food), and, in the case of DHA in supplements, galenic form (microencapsulation, emulsification, etc) (Schuchardt and Hahn, 2013). Of course, bioavailability entails the transport of DHA (as FFA or MAG) to the lymph and the blood. For this transport to occur, DHA is re-esterified to TAG (rTAG) in the endoplasmic reticulum of enterocytes (Schuchardt and Hahn, 2013). Afterwards, these rTAG molecules, along with cholesteryl esters and PL, form chylomicrons with apolipoproteins. These chylomicrons are released into the lymph and enter the bloodstream. All these processes may affect DHA bioavailability.

It has been observed in *in vivo* studies with humans that DHA bound in TAG is more bioavailable than DHA bound in EE form (Dyerberg et al., 2010; Neubronner et al., 2011). The latter form is found sometimes in DHA supplements. A first explanation for this difference may be found in *in vitro* studies showing that pancreatic lipases hydrolyze EEs 10 to 50 times less efficiently than glycerol esters in TAG (Yang et al., 1990a). Another reason may be differences in the re-esterification of DHA to TAG after absorption. This process requires glycerol and 2-MAG, which are readily available when DHA is bound in TAG, but absent when DHA is bound in the EE form, since there is no release of glycerol during digestion in this case. Accordingly,

re-esterification may be delayed due to difficulties in providing the missing glycerol (Yang et al., 1990b).

In krill oil, which is rich in PL, it has been claimed that its DHA bioavailability is higher on the basis of *in vivo* studies (Maki et al., 2009; Schuchardt et al., 2011). This higher bioavailability may be related to the high share of PL. Indeed, it is known that PL are amphiphilic and present emulsifying properties. Particularly, it has been shown that PL from lecithin (containing PC) alters the surface structure of oil droplets in such a way that the pancreatic lipases are able to readily access their substrate (Mun et al., 2007). PL are also involved in the formation of mixed micelles, which contribute to higher lipid absorption. Moreover, it has been hypothesized that phospholipases can be quite efficient in coming into close proximity to the emulsified lipids (Schuchardt and Hahn, 2013). However, there are also authors (Köhler et al., 2015) upholding that PL is not necessarily better absorbed than TAG. Taking into account that FFA also exhibit a high bioavailability (Dyerberg et al., 2010; Kling et al., 2011) because no chemical bond needs to be broken and that rTAG seem to have a bioavailability higher than natural fish oil (TAG) and FFA (Dyerberg et al., 2010), it can be put forward a possible DHA bioavailability order: DHA-rTAG > DHA-TAG ~ DHA-FFA > DHA-EE, with the position of DHA-PL above that of DHA-TAG. However, more scientific research is necessary for proving this order, especially concerning a supposed higher bioavailability of PL with respect to TAG and even rTAG.

8 -- Conclusions

DHA is a key nutritional n-3 PUFA and needs to be supplied by the human diet, among many reasons, because there is evidence suggesting an important role of dietary DHA in the development of the neurological system. Indeed, DHA is found in significant amounts in the retinal and neuronal cell membranes due to its high fluidity. Data seem to support a multifarious action of DHA through more than a single mechanism on multiple neurological health endpoints. In particular, regarding autism disorders, high DHA intake may be an important component in disease prevention. However, more studies are required to strengthen the connection between autism and dietary DHA, including a detailed analysis involving the determination of the DHA content in each lipid group. In what concerns behavioural disorders, the evidence is also

contradictory, thereby raising the need of further studies. From all screened studies on autism, ADHD, and other disorders, it can be concluded that study samples should be larger for greater statistical significance and RCTs should be more carefully designed. Besides, DHA bioaccessibility in seafood and supplements, taking into account the specific chemical binding form, and the gut-brain axis need to be regarded as crucial issues in all studies. Accordingly, after weighing observational and RCT studies, it can be stated that maternal intake of DHA during pregnancy and breastfeeding may be beneficial for the healthy development of children. An average daily intake of, at least, 200 mg of DHA in the mother's diet can be endorsed as a balanced recommendation on the basis of available information. Moreover, there is some evidence arguing for the importance of keeping high DHA intake after birth during the first critical years of child's brain development.

Disclosure Statement

There is no conflict of interest.

Acknowledgments

This work was supported by the following Post Doctoral Grants: Ref.: SFRH/BPD/102689/2014 ("Fundação para a Ciência e a Tecnologia", FCT) for the author Carlos Cardoso; Ref.: SFRH/BPD/64951/2009 (FCT) and DIVERSIAQUA (MAR2020) for the author Cláudia Afonso.

References

Ackman, R. G., Ratnayake, W. M. N., and Olsson, B. (1988). The “basic” fatty acid composition of Atlantic fish oils: potential similarities useful for enrichment of polyunsaturated fatty acids by urea complexation. *JAOCS* **65**: 136--138.

Afonso, C., Cardoso, C., Lourenço, H. M. Anacleto, P., Bandarra, N. M., Carvalho, M. L., Castro, M., and Nunes, M. L. (2013a). Evaluation of hazards and benefits associated with the consumption of six fish species from the Portuguese coast. *J. Food Comp. Anal.* **32**: 59-67.
Afonso, C., Lourenço, H. M., Cardoso, C., Bandarra, N. M., Carvalho, M. L., Castro, M., and Nunes, L. (2013b). From fish chemical characterisation to the benefit-risk assessment -- Part A. *Food Chem.* **137**: 99-107.

Afonso, C., Costa, S., Cardoso, C., Bandarra, N. M., Batista, I., Coelho, I., Castanheira, I., and Nunes, M. L. (2015). Evaluation of the risk/benefit associated to the consumption of raw and cooked farmed meagre based on the bioaccessibility of selenium, eicosapentaenoic acid and docosahexaenoic acid, total mercury, and methylmercury determined by an *in vitro* digestion model. *Food Chem.* **170**: 249-256.

Akanbi, T. O., Sinclair, A. J., and Barrow, C. J. (2014). Pancreatic lipase selectively hydrolyses DPA over EPA and DHA due to location of double bonds in the fatty acid rather than regioselectivity. *Food Chem.* **160**: 61--66.

Al-Farsi, Y. M., Waly, M. I., Deth, R. C., Al-Sharbati, M. M., Al-Shafaee, M., Al-Farsi, O., Al-Khaduri, M. M., Al-Adawi, S., Hodgson, N. W., Gupta, I., and Ouhtit, A. (2013). Impact of nutrition on serum levels of docosahexaenoic acid among Omani children with autism. *Nutrition* **29(9)**: 1142-1146.

Amminger, G. P., Berger, G. E., Schafer, M. R., Klier, C., Friedrich, M. H., and Feucht, M. (2007). Omega-3 fatty acids supplementation in children with autism: a double-blind randomized, placebo-controlled pilot study. *Biological Psychol.* **61**: 551--553

Anderson, G. J. (1994). Developmental sensitivity of the brain to dietary n-3 fatty acids. *J. Lipid Res.* **35**: 105-111.

Anderson, G. J., and Connor, W. E. (1994). Accretion of n-3 fatty acids in the brain and retina of chick fed a low linolenic acid diet supplemented with docosahexaenoic acid. *Am. J. Clin. Nutr.* **59(6)**: 1338-1346.

Anderson, P., and Doyle, L. W. (2003). Victorian Infant Collaborative Study Group. Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *JAMA* **289**: 3264--3272.

Anderson, P. J., and Doyle, L. W. (2004). Victorian Infant Collaborative Study Group. Executive functioning in school-aged children who were born very preterm or with extremely low birth weight in the 1990s. *Paediatrics* **114**: 50--57.

Anderson, V., Jacobs, R., and Anderson, P. (2008). *Executive functions and the frontal lobes. A lifespan perspective*. Taylor and Francis: New York, USA.

Bandarra, N. M., Batista, I., Nunes, M. L., Empis, J. M., and Christie, W. W. (1997). Seasonal changes in lipid composition of sardine (Sardina pilchardus). J. Food Sci. ***62(1)****: 40-42.*

Bandarra, N.M., Calhau, M. A., Oliveira, L., Ramos, M., Dias, M. G., Bartolo, H., Faria, M. R., Fonseca, M. C., Gonçalves, J., Batista, I., and Nunes, M. L. (2004). Composição e valor nutricional dos produtos da pesca mais consumidos em Portugal. *Publicações Avulsas IPIMAR* **11**: 1-103.

Bandarra, N. M., Cardoso, C., Costa, S., and Afonso, C. (2016). Bioaccessibility of n-3 PUFA from seafood: Fish *vs* supplements: A case study. Presented in the 14th Euro Fed Lipid Congress, Ghent, September 18 -- September 21st 2016.

Bazan, N. G., Birkle, D. L., and Reddy, T. S. (1984). Docosahexaenoic acid (22:6, n-3) is metabolized to lipoxygenase reaction products in the retina. *Biochem. Biophys. Res. Comm.* **125**: 741--747.

Bazan, N. G., Molina, M. F., and Gordon, W. C. (2011). Docosahexaenoic acid signalolipidomics in nutrition: Significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases. Ann. Rev. Nutr. ***31****: 321-351.*

Bell, J. G., MacKinlay, E. E., Dick, J. R., MacDonald, D. J., Boyle, R. M., and Glen, A. C. A. (2004). Essential fatty acids and phospholipase A2 in autistic spectrum disorders. *Prostaglandins Leukotrienes Essential Fatty Acids* **71(4)**: 201--204.

Bernardi, J. R., Escobar, R. S., Ferreira, C. F., and Silveira, P. P. (2012). Fetal and neonatal levels of omega-3: effects on neurodevelopment, nutrition, and growth. *Sci, World J.* **2012**: 202473.

Bhutta, A. T., Cleves, M. A., Casey, P. H., Cradock, M. M., and Anand, K. J. (2002). Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *JAMA* **288(6)**: 728--737.

Blasbalg, T. L., Hibbeln, J. R., Ramsden, C. E., Majchrzak, S. F., and Rawlings, R. R. (2011). Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am. J. Clin. Nutr.* **93**: 950--962.

Bloch, M. H., and Qawasmi, A. (2011). Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder sympto- matology: Systematic review and meta-analysis. *J. Am. Acad. Child Adolesc. Psych.* **50**: 991-1000.

Boucher, O., Burden, M. J., Muckle, G., Saint-Amour, D., Ayotte, P., Dewailly, E., Nelson, C. A., Jacobson, S. W., and Jacobson, J. L. (2011). Neurophysiologic and neurobehavioral evidence of beneficial effects of prenatal omega-3 fatty acid intake on memory function at school age. Am. J. Clin. Nutr. 93: 1025--1037.

Bradshaw, J. L. (2001). Developmental disorders of the frontostriatal system, neuropsychological, neuropsychiatric and evolutionary perspectives. Psychology Press: Philadelphia, USA.

Brand, A., Schonfeld, E., Isharel, I., and Yavin, E. (2008). Docosahexaenoic acid-dependent iron accumulation in oligodendroglia cells protects from hydrogen peroxide-induced damage. *J. Neurochem.* **105**: 1325--1335.

Brown, C. M., and Austin, D. W. (2011). Autistic disorder and phospholipids: A review. *Prostaglandins Leukotrienes Essential Fatty Acids*, **84(1-2)**: 25-30.

Bu, B., Ashwood, P., Harvey, D., King, I. B., Van de Water, J., and Jin, L. W. (2006). Fatty acid compositions of red blood cell phospholipids in children with autism. *Prostaglandins Leukotrienes Essential Fatty Acids*, **74(4)**: 215-221.

Burdge, G. C., and Wootton, S. A. (2002). Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.* **88**: 411--420.

*Burri, L. (2015). Chapter 96 -- Krill oil supplementation and cognitive function. In: Diet and Nutrition in Dementia and Cognitive Decline, Martin, C. R. and Preedy, V. (ed.), pp. 1031-1038. Academic Press: Oxford, UK (*ISBN: 978-0-12-407824-6).

Calandria, J. M., Marcheselli, V. L., Mukherjee, P. K., Uddin, J., Winkler, J. W., Petasis, N. A., and Bazan, N. G. (2009). Selective survival rescue in 15-lipoxygenase-1-deficient retinal pigment epithelial cells by the novel docosahexaenoic acid-derived mediator, neuroprotectin D1. *J. Biol. Chem.* **284(26)**: 17877--17882.

*Camargos, A. C. R., Mendonça, V. A., Andrade, C. A., Oliveira, K. S. C., and Lacerda, A. C. R. (2016). Overweight and obese infants present lower cognitive and motor development scores than normal-weight peers. Res. Developm. Disabilities **59**: 410-416.*

Campoy, C., Escolano-Margarit, M. V., Ramos, R., Parrilla, M., Beyer, J., Csábi, G., Pérez-García, M., Gil, A., Decsi, T., and Koletzko, B. (2010). Analysis of long term effects of fish oil and 5-MTHF supplementation to pregnant women on neurological outcome of their offspring: The Nuheal trial. *J. Ped. Gastroenterol. Nutr.* **50**: E23--E24.

*Cansev, M., and Wurtman, R. J. (2007). Chronic administration of docosahexaenoic acid or eicosapentaenoic acid, but not arachidonic acid, alone or in combination with uridine, increases brain phosphatide and synaptic protein levels in gerbils. Neurosci. **148**: 421-431.*

Cardoso, C., Afonso, C., Lourenço, H., Costa, S., and Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk-benefit evaluation of food. *Trends Food Sci. Technol.* **41**: 5-23.

Carlson, S. E., Werkman, S. H., Peeples, J. M., and Wilson, W. M. (1994). Long-chain fatty acids and early visual and cognitive development of preterm infants. *Eur. J. Clin. Nutr.* **48(Suppl. 2)**: S27-S30.

Chugani, D. C., Muzik, O., Behen, M., Rothermel, R., Janisse, J. J., Lee, J., and Chugani, H. T. (1999). Developmental changes in brain serotonin synthesis capacity in autistic and nonautistic children. *Ann. Neurol.* **45**: 287--295.

Clark-Taylor, T., and Clark-Taylor, B. E. (2004). Is autism a disorder of fatty acid metabolism? Possible dysfunction of mitochondrial □-oxidation by long chain acyl-CoA dehydrogenase. Medical Hypoth. ***62(6)****: 970-975.*

Colombo, J., Kannass, K. N., Shaddy, D. J., Kundurthi, S., Maikranz, J. M., Anderson, C. J., Blaga, O. M., and Carlson, S. E. (2004). Maternal DHA and the development of attention in infancy and toddlerhood. *Child Developm.* **75(4)**: 1254--1267.

Colter, A. L., Cutler, C., and Meckling, K. A. (2008). Fatty acid status and behavioural symptoms of attention deficit hyperactivity disorder in adolescents: a case control study. *Nutr. J.* **7**: 8.

Conquer, J. A., and Holub, B. J. (1997). Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. *Lipids* **32(3)**: 341-345.

Cooper, R. E., Tye, C., Kuntsi, J., Vassos, E., and Asherson, P. (2016). The effect of omega-3 polyunsaturated fatty acid supplementation on emotional dysregulation, oppositional behaviour and conduct problems in ADHD: A systematic review and meta-analysis. *J. Affective Disorders* **190**: 474-482.

Cordain, L., Eaton, S. B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B. A., O'Keefe, J. H., and Brand-Miller, J. (2005). Origins and evolution of the Western diet: health implications for the 21st century. *Am. J. Clin. Nutr.* **81**: 341--354.

Costa, S., Afonso, C., Cardoso, C., Batista, I., Chaveiro, N., Nunes, M. L., and Bandarra, N. M. (2015). Fatty acids, mercury, and methylmercury bioaccessibility in salmon (*Salmo salar*) using an *in vitro* model: Effect of culinary treatment. *Food Chem.* **185**: 268-276.

Costa, S., Afonso, C., Cardoso, C., Oliveira, R., Alves, F., Nunes, M. L., and Bandarra, N. M. (2016). Towards a deeper understanding of fatty acid bioaccessibility and its dependence on culinary treatment and lipid class: A case study of gilthead seabream (*Sparus aurata*). *Br. J. Nutr.* **116**: 1816-1823.

*Cunnane, S. C., Chouinard-Watkins, R., Castellano, C. A., and Barberger-Gateau, P. (2013). Docosahexaenoic acid homeostasis, brain aging and Alzheimer's disease: Can we reconcile the evidence? Prostaglandins Leukotrienes Essential Fatty Acids **88**: 61-70.*

Dadorama, S. (1996). Amino acids, peptides, and proteins. In: *Food Chemistry* Fennema, O. R. (ed.), pp. 321--429. Marcel Dekker Inc.: New York, USA (ISBN: 978-0824796914).

Decsi, T., Campoy, C., and Koletzko, B. (2005). Effect of n-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial. *Ad. Exp. Med. Biol.* **569**: 109--113.

de La Presa Owens, S., and Innis, S. M. (1999). Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotoninergic neurotransmitters in frontal cortex caused by a linoleic and alpha-linolenic acid deficient diet in formula-fed piglets. *J. Nutr.* **129**: 2088--2093.

Delorme, R., Ey, E., Toro, R., Leboyer, M., Gillberg, C., and Bourgeron, T. (2013). Progress toward treatments for synaptic defects in autism. *Nature Med.* **19**: 685--694.

Dufault, R., Schnoll, R., Lukiw, W. J., Leblanc, B., Cornett, C., Patrick, L., Wallinga, D., Gilbert, S. G., and Crider, R. (2009). Mercury exposure, nutritional deficiencies and metabolic disruptions may affect learning in children. *Behav. Brain Functions* **5**:44.

Dunstan, J. A., Simmer, K., Dixon, G., and Prescott, S. L. (2008). Cognitive assessment of children at 2(1/2) years following fish oil supplementation in pregnancy: a randomized controlled trial. *Arch. Disease Childhood. Fetal Neonatal Ed.* **93(1)**: F45--F50.

Durand, T., De Felice, C., Signorini, C., Oger, C., Bultel-Poncé, V., Guy, A., Galano, J. M., Leoncini, S., Ciccoli, L., Pecorelli, A., Valacchi, G., and Hayek, J. (2013). F2-dihomo-isoprostanes and brain white matter damage in stage 1 Rett syndrome. *Biochimie* **95**: 86-90.

Dyerberg, J., Madsen, P., Møller, J. M., Aardestrup, I., and Schmidt, E. B. (2010). Bioavailability of marine n-3 fatty acid formulations. *Prostaglandins Leukotrienes Essential Fatty Acids* **83**: 137-141.

*Editorial (2015). Neuro-nutraceuticals: The path to brain health via nourishment is not so distant. Neurochem. Int. **89**: 1-6.*

Escolano-Margarit, M. V., Ramos, R., Beyer, J., Csábi, G., Parrilla-Roure, M., Cruz, F., Perez-Garcia, M., Hadders-Algra, M., Gil, A., Decsi, T., Koletzko, B. V., and Campoy, C. (2011). Prenatal DHA status and neurological outcome in children at age 5.5 years are positively associated. *J. Nutr.* **141(6)**: 1216--1223.

Farooqui, A. A., and Horrocks, L. A. (2001). Plasmalogens: workhorse lipids of membranes in normal and injured neurons and glia. *Neuroscientist* **7(3)**: 232--245.

*Freemantle, E., Vandal, M., Tremblay-Mercier, J., Tremblay, S., Blachère, J. C., Bégin, M. E., Brenna, J. T., Windust, A., and Cunnane, S. C. (2006). Omega-3 fatty acids, energy substrates, and brain function during aging. Prostaglandins Leukotrienes Essential Fatty Acids **75**: 213-220.*

Gale, C. R., Marriott, L. D., Martyn, C. N., Limond, J., Inskip, H. M., Godfrey, K. M., Law, C. M., Cooper, C., West, C., Robinson, S. M., and Group for Southampton Women's Survey Study (2010). Breastfeeding, the use of docosahexaenoic acid-fortified formulas in infancy and neuropsychological function in childhood. *Arch. Disease Childhood* 95: 174-179.

Gao, J., Wang, X., Sun, H., Cao, Y., Liang, S., Wang, H., Wang, Y., Yang, F., Zhang, F., and Wu, L. (2016). Neuroprotective effects of docosahexaenoic acid on hippocampal cell death and learning and memory impairments in a valproic acid-induced rat autism model. Int. J. Developm. Neurosci. ***49****: 67-78.*

Gaposchkin, D. P., and Zoeller, R. A. (1999). Plasmalogen status influences docosahexaenoic acid levels in a macrophage cell line: insights using ether lipid-deficient variants. *J. Lipid Res.* **40**: 495--503.

Glomset, J. A. (2006). Role of docosahexaenoic acid in neuronal plasma membranes. Science's STKE ***321****: pe6.*

Gould, J. F., Smithers, L. G., and Makrides, M. (2013). The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **9**: 531--544.

Gould, J. F., Treyvaud, K., Yelland, L. N., Anderson, P. J., Smithers, L. G., Gibson, R. A., McPhee, A. J., and Makrides, M. (2016). Does n-3 LCPUFA supplementation during pregnancy increase the IQ of children at school age? Follow-up of a randomised controled trial. BMJ Open ***6****: e011465.*

Gow, R. V. and Hibbeln, J. R. (2014). Omega-3 fatty acid and nutrient deficits in adverse neurodevelopment and childhood behaviors. Child Adolesc. Psych. Clin. North Am. ***23(3)****: 555-590.*

Grantham-McGregor, S., Cheung, Y. B., Cueto, S., Glewwe, P., Richter, L., and Strupp, B. (2007). Developmental potential in the first 5 years for children in developing countries. *Lancet* **369**: 60--70.

Guesnet, P., and Alessandri, J. M. (2011). Docosahexaenoic acid (DHA) and the developing central nervous system (CNS) --- implications for dietary recommendations. *Biochimie* **93**: 7--12.

Guo, M., Stockert, L., Akber, M., and Kim, H. Y. (2007). Neuronal specific increase of phosphatidylserine by docosahexaenoic acid. *J. Mol. Neurosci.* **33(1)**: 67-73.

Haggarty, P., Page, K., Abramovich, D. R., Ashton, J., and Brown, D. (1997). Long-chain polyunsaturated fatty acid transport across the perfused human placenta. *Placenta* **18**: 635--642.

Hamazaki, K., Syafruddin, D., Tunru, I. S., Azwir, M. F., Asih, P. B., Sawazaki, S., and Hamazaki, T. (2008). The effects of docosahexaenoic acid-rich fish oil on behavior, school attendance rate and malaria infection in school children---A double-blind, randomized, placebo-controlled trial in Lampung, Indonesia. Asia Pac. J. Clin. Nutr. 17(2): 258--263.

Hamazaki, T., and Hamazaki, K. (2008). Fish oils and aggression or hostility. *Prog. Lipid Res.* **47**: 221--232.

Hamilton, J. A., and Brunaldi, K. (2007). A model for fatty acid transport into the brain. *J. Mol. Neurosci.* **33(1)**: 12--17.

*Hanhoff, T., Lücke, C., and Spener, F. (2002). Insights into binding of fatty acids by fatty acid binding proteins. Mol. Cell. Biochem. **239(1-2)**: 45-54.*

Harris, W. S., Poston, W. C., and Haddock C. K. (2007). Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. *Atherosclerosis* **193**: 1--10.

Hashimoto, K., Iwata, Y., Nakamura, K., Tsujii, M., Tsuchiya, K. J., Sekine, Y., Suzuki, K., Minabe, Y., Takei, N., Iyo, M., Mori, N. (2006). Reduced serum levels of brain-derived neurotrophic factor in adult male patients with autism. *Prog. Neuro-Psychopharmacol. Biol. Psych.* **30(8)**: 1529-1531.

Helland, I. B., Smith, L., Saarem, K., Saugstad, O. D., and Drevon, C. A. (2003). Maternal supplementation with very-long-chain n−3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics* **111**: 39--44.

Helland, I. B., Smith, L., Blomén, B., Saarem, K., Saugstad, O. D., and Drevon, C. A. (2008). Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics* **122(2)**: 472--479.

Hellhammer, J., Hero, T., Franz, N., Contreras, C., and Schubert, M. (2012). Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. *Nutr. Res.* **32**: 241-250.

Hibbeln, J. R., and Davis, J. M. (2009). Considerations regarding neuropsychiatric nutritional requirements for intakes of omega-3 highly unsaturated fatty acids. *Prostaglandins Leukotrienes Essential Fatty Acids* **81**: 179-186.

Hirayama, S., Hamazaki, T., and Terasawa, K. (2004). Effect of docosahexaenoic acid-containing food administration on symptoms of attention-deficit/hyperactivity disorder-a placebo-controlled double blind study. *Eur. J. Clin. Nutr.* **58**: 467-473.

*Igarashi, M., DeMar, J. J. C., Ma, K., Chang, L., Bell, J. M., and Rapoport, S. I. (2007). Docosahexaenoic acid synthesis from □-linolenic acid by rat brain is unaffected by dietary n-3 PUFA deprivation. J. Lipid Res. **48**: 1150-1158.*

Itomura, M., Hamazaki, K., Sawazaki, S., Kobayashi, M., Terasawa, K., Watanabe, S., and Hamazaki, T. (2005). The effect of fish oil on physical aggression in schoolchildren---A randomized, double-blind, placebo-controlled trial. J. Nutr. Biochem. 16: 163--171.

Janssen, C. I., and Kiliaan, A. J. (2014). Long-chain polyunsaturated fatty acids (LCPUFA) from genesis to senescence: the influence of LCPUFA on neural development, aging, and neurodegeneration. *Prog. Lipid Res.* **53**: 1-17.

Johnson, M. H. (2001). Functional brain development in humans. *Nature Rev. Neuroscience* **2**: 475--483.

Johnston, M., Landers, S., Noble, L., Szucs, K., and Viehmann, L. for the Section on Breastfeeding (2012). Breastfeeding and the use of human milk. *Pediatrics* **129**: e827-e841.

*Jory, J. (2016). Abnormal fatty acids in Canadian children with autism. Nutrition **32**: 474-477.*

Judge, M. P., Harel, O., and Lammi-Keefe, C. J. (2007a). Maternal consumption of a DHA-functional food during pregnancy: comparison of infant performance on problem-solving and recognition memory tasks at 9 months of age. *Am. J. Clin. Nutr.* **85**: 1572--1577.

Judge, M. P., Harel, O., and Lammi-Keefe, C. J. (2007b). A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids* **42**: 117--122.

*Judge, M. P., Cong, X., Harel, O., Courville, A. B., and Lammi-Keefe, C. J. (2012). Maternal consumption of a DHA-containing functional food benefits infant sleep patterning: An early neurodevelopmental measure. Early Human Development **88**: 531-537.*

Kahya, N., Scherfeld, D., Bacia, K., Poolman, B., and Schwille, P. (2003). Probing lipid mobility of raft-exhibiting model membranes by fluorescence correlation spectroscopy. *J. Biol. Chem.* **278**: 28109-28115.

Kannass, K. N., Colombo, J., and Carlson, S. E. (2009). Maternal DHA levels and toddler free-play attention. *Developm. Neuropsychol.* **34**: 159--174.

*Kidd, P. M. (2002). Autism, an extreme challenge to integrative medicine. Part 1: The knowledge base. Alt. Med. Rev. **7**: 292-316.*

Kling, D. F., Johnson, J., Rooney, M,. and Davidson, M. (2011). Omega-3 free fatty acids demonstrate more than 4-fold greater bioavailability for EPA and DHA compared with omega-3-acid ethyl esters in conjunction with a low-fat diet: the ECLIPSE study. *J. Clin. Lipidol.* **5(3)**: 231.

*Klose, C., Surma, M. A., and Simons, K. (2013). Organellar lipidomics -- background and perspectives. Curr. Op. Cell Biol. **25**: 406-413.*

Köhler, A., Sarkkinen, E., Tapola, N., Niskanen, T., and Bruheim, I. (2015). Bioavailability of fatty acids from krill oil, krill meal and fish oil in healthy subjects --a randomized, single-dose, cross-over trial. *Lipids Health Disease* **14**: 19-28.

LeCluyse, E. L., Witek, R. P., Andersen, M. E., and Powers, M. J. (2012). Organotypic liver culture models: meeting current challenges in toxicity testing. *Crit. Rev. Toxicol.* **42(6)**: 501-548.

*Lee, A. G. (2011). Biological membranes: the importance of molecular detail. Trends Biochem. Sciences **36(9)**: 493-500.*

Levitt, P.(2003). Structural and functional maturation of the developing primate brain. *J. Pediatrics* **143**: 35--45.

Lombard, J. (1998). Autism: a mitochondrial disorder? *Medical Hypoth.* **50**: 497--500.

Lozoff, B., and Georgieff, M. K. (2006). Iron deficiency and brain development. *Seminars Pediatric Neurol.* **13**: 158--165.

Lukiw, W. J., Cui, J., Marcheselli, V. L., Bodker, M., Botkjaer, A., Gotlinger, K., Serhan, C. N., and Bazan, N. G. (2005). A role for docosahexaenoic acidderived neuroprotectin D1 in neural cell survival and Alzheimer disease. *J. Clin. Inv.* **115(10)**: 2774--2783.

Lukiw, W. J., and Bazan, N. G. (2008). Docosahexaenoic acid and the aging brain. *J. Nutr.* **138(12)**: 2510-2514.

Maekawa, M., Iwayama, Y., Arai, R., Nakamura, K., Ohnishi, T., Toyota, T., Tsujii, M., Okazaki, Y., Osumi, N., Owada, Y., Mori, N., and Yoshikawa, T. (2010). Polymorphism screening of brain-expressed FABP7, 5 and 3 genes and association studies in autism and schizophrenia in Japanese subjects. *J. Human Gen.* **55(2)**: 127--130.

Maki, K. C., Reeves, M. S., Farmer, M., Griinari, M., Berge, K., Vik, H., Hubacher, R., and Rains, T. M. (2009). Krill oil supplementation increases plasma concentrations of eicosapentaenoic and docosahexaenoic acids in overweight and obese men and women. *Nutr. Res.* **29**: 609--615.

*Makri, A., Bellou, S., Birkou, M., Papatrehas, K., Dolapsakis, N. P., Bokas, D., Papanikolaou, S., Aggelis, G. (2011). Lipid synthesized by micro-algae grown in laboratory- and industrial-scale bioreactors. Eng. Life Sciences **11(1)**: 52-58.*

Malkova, N. V., Yu, C. Z., Hsiao, E. Y., Moore, M. J., and Patterson, P. H. (2012). Maternal immune activation yields offspring displaying mouse versions of the three core symptoms of autism. *Brain Behavior Imm.* **26**: 607--616.

Mankad, D., Dupuis, A., Smile, S., Roberts, W., Brian, J., Lui, T., Genore, L., Zaghloul, D., Iaboni, A., Marcon, P. M. A., and Anagnostou, E. (2015). A randomized, placebo controlled trial of omega-3 fatty acids in the treatment of young children with autism. Mol. Autism ***6****: 18.*

Martinez M. (1992). Tissue levels of polyunsaturated fatty acids during early human development. *J. Pediatrics* **120**: S129-138.

Martínez. M., and Mougan, I. (1998). Fatty acid composition of human brain phospholipids during normal development. J. Neurochem. ***71****: 2528-2533.*

McCann, J. C., and Ames, B. N. (2005). Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. Am. J. Clin. Nutr. ***82****: 281-295.*

Meguid, N. A., Atta, H. M., Gouda, A. S., and Khalil, R. O. (2008). Role of polyunsaturated fatty acids in the management of Egyptian children with autism. *Clin. Biochem.* **41**: 1044--1048.

Meiri, G., Bichovsky, Y., and Belmaker, R. H. (2009). Omega 3 fatty acid treatment in autism. *J. Child Adolesc. Psychopharmacol.* **19**: 449--451.

Meldrum, S., Dunstan, J. A., Foster, J. K., Simmer, K., and Prescott, S. L. (2015). Maternal fish oil supplementation in pregnancy: a 12 year follow-up of a randomized controlled trial. *Nutrients* **7(3)**: 2061--2067.

Milte, C. M., Parletta, N., Buckley, J. D., Coates, A. M., Young, R. M., and Howe, P. R. C. (2012). Eicosapentaenoic and docosahexaenoic acids, cognition, and behavior in children with attention-deficit/hyperactivity disorder: A randomized controlled trial. Nutrition ***28****: 670-677.*

Milte, C. M., Parletta, N., Buckley, J. D., Coates, A. M., Young, R. M., and Howe, P. R. (2015). Increased erythrocyte eicosapentaenoic acid and docosahexaenoic acid are associated with improved attention and behavior in children with ADHD in a randomized controlled three-way crossover trial. *J. Attention Disorders* **19(11)**: 954--964.

Minoo, P., Moyer, M. P., and Jass, J. R. (2007). Role of BRAF-V600E in the serrated pathway of colorectal tumourigenesis. *J. Pathol.* **212(2)**: 124-133.

Mitchell, E. A., Aman, M. G., Turbott, S. H., and Manku, M. (1987). Clinical characteristics and serum essential fatty acid levels in hyperactive children. *Clin. Pediatrics (Phila)* **26(8)**: 406-411.

Mitchell, R. W., and Hatch, G. M. (2011). Fatty acid transport into the brain: of fatty acid fables and lipid tails. *Prostaglandins Leukotrienes Essential Fatty Acids* **85(5)**: 293--302.

*Mostafa, G. A., El-Khashab, H. Y., and Al-Ayadhi, L. Y. (2015). A possible association between elevated serum levels of brain-specific auto-antibodies and reduced plasma levels of docosahexaenoic acid in autistic children. J. Neuroimmunol. **280**: 16-20.*

Müller, C. P., Reichel, M., Mühle, C., Rhein, C., Gulbins, E., and Kornhuber, J. (2015). Brain membrane lipids in major depression and anxiety disorders. *Biochim. Biophys. Acta* **1851(8)**: 1052-1065.

Mun, S., Decker, E. A., and McClements, D. J. (2007). Influence of emulsifier type on *in vitro* digestibility of lipid droplets by pancreatic lipase. *Food Res. Int.* **40**: 770--781.

Neubronner, J., Schuchardt, J. P., Kressel, G., Merkel, M., von Schacky, C., and Hahn, A. (2011). Enhanced increase of omega-3 index in response to long-term n−3 fatty acid supplementation from triacylglycerides versus ethyl esters. *Eur. J. Clin. Nutr.* **65**: 247--254.

Nyaradi, A., Li, J., Hickling, S., Foster, J., and Oddy, W. H. (2013). The role of nutrition in children's neurocognitive development, from pregnancy through childhood. *Front. Human Neurosci.* **7(97)**: 1-16.

Nunes, M. L., Bandarra, N., Oliveira, L., Batista, I., and Calhau, M. A. (2006). Composition and nutritional value of fishery products consumed in Portugal. **In** *Seafood research from fish to dish. Quality, safety and processing of wild and farmed fish*, Luten, J. B., Jacobsen, C., Bekaert, K., Saebø, A., Oehlenschläger, J. (Eds.), pp. 477-487. Wageningen Academic Publishers: Wageningen, The Netherlands (ISBN: 978-90-8686-005-0).

Orr, S. K., Palumbo, S., Bosetti, F., Mount, H. T., Kang, J. X., Greenwood, C. E., Ma, D. W., Serhan, C. N., and Bazinet, R. P. (2013). Unesterified docosahexaenoic acid is protective in neuroinflammation. *J. Neurochem.* **127**: 378--393.

*Osendarp, S. J. M. (2011). The role of omega-3 fatty acids in child development. OCL **18(6)**: 307-313.*

Pardo, C. A., and Eberhart, C. G. (2007). The neurobiology of autism. *Brain Pathol.* **17**: 434--447.

*Patil, V., Källqvist, T., Olsen, E., Vogt, G., and Gislerød, H. R. (2007). Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquaculture Int. **15**: 1-9.*

Paustenbach, D. J. (2000). The practice of exposure assessment: a state-of-the-art review (Reprinted from Principles and Methods of Toxicology, 4th edition, 2001). *J. Toxicol. Env. Health Part B -- Crit. Rev.* **3**: 179-291.

*Peinado, I., Girón, J., Koutsidis, G., and Ames, J. M. (2014). Chemical composition, antioxidante activity and sensory evaluation of five different species of brown edible seaweeds. Food Res. Int. **66**: 36-44.*

*Plourde, M., and Cunnane, S. C. (2007). Extremely limited synthesis of long chain polyunsaturates in adults: implications for their dietary essentiality and use as supplements. App. Physiol. Nutr. Met. **32(4)**: 619-634.*

*Porta, A., Pasi, M., Brunoldi, E., Zanoni, G., and Vidari, G. (2013). Biology and chemistry of neuroprostanes. First total synthesis of 17-A_4-NeuroP: Validation of a convergent strategy to a number of cyclopentenone neuroprostanes. Chem. Phys. Lipids **174**: 64-74.*

Rapoport, S. I., Rao, J. S., and Igarashi, M. (2007). Brain metabolism of nutritionally essential polyunsaturated fatty acids depends on both the diet and the liver. *Prostaglandins Leukotrienes Essential Fatty Acids* **77**: 251--61.

Rapoport, S. I. (2013). Translational studies on regulation of brain docosahexaenoic acid (DHA) metabolism *in vivo*. *Prostaglandins Leukotrienes Essential Fatty Acids* **88(1)**: 79-85.

Ratajczak, H. V. (2011). Theoretical aspects of autism: Causes -- A review. J. Immunotoxicol. ***8(1)****: 68-79.*

Ratnayake, W. M. N., and Hollywood, R. (1997). Influence of diet on human milk fatty acid composition. *Abstracts, 88th AOCS Annual Meeting and Expo*: 96.

Richardson, A., and Ross, M. (2000). Fatty acid metabolism in neurodevelopmental disorder: a new perspective on associations between attention-deficit/hyperactivity disorder, dyslexia, dyspraxia and the autistic spectrum. *Prostaglandins Leukotrienes Essential Fatty Acids* **63(1-2)**: 1--9.

Richardson, A. J., Burton, J. R., Sewell, R. P., Spreckelsen, T. F., and Montgomery, P. (2012). Docosahexaenoic acid for reading, cognition and behavior in children aged 7--9 years: A randomized, controlled trial (the DOLAB Study). *PLoS One* **7(9)**: e43909.

Ross, B. M. (2009). Omega-3 polyunsaturated fatty acids and anxiety disorders. *Prostaglandins Leukotrienes Essential Fatty Acids* **81**: 309--312.

Sawazaki, S., Salem, N. J., and Kim, H. Y. (1994). Lypoxigenation of docosahexaenoic acid by the rat pineal body. J. Neurochem. ***62(6)****: 2437-2447.*

Schendel, D., and Karapurkar, B. T. (2008). Birth weight and gestational age characteristics of children with autism, including a comparison with other developmental disabilities. *Pediatrics* **121(6)**: 1155-1164.

Schuchardt, J. P., Huss, M., Stauss-Grabo, M., and Hahn, A. (2010). Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur. J. Pediatrics* **169**: 149-164.

Schuchardt, J. P., Schneider, I., Meyer, H., Neubronner, J., von Schacky, C., and Hahn, A. (2011). Incorporation of EPA and DHA into plasma phospholipids in response to different omega-3 fatty acid formulations ---a comparative bioavailability study of fish oil vs. krill oil. *Lipids Health Disease* **10**: 145.

Schuchardt, J. P., and Hahn, A. (2013). Bioavailability of long-chain omega-3 fatty acids. *Prostaglandins Leukotrienes Essential Fatty Acids* **89**: 1--8.

Schumann, K., Classen, H. G., Hages, M., Prinz-Langenhol, R., Pietrzik, K., and Biesalski, H. K. (1997). Bioavailability of oral vitamins, minerals and trace elements in perspective. *Drug Res.* **47**: 369-380.

Schwartzer, J. J., Careaga, M., Onore, C. E., Rushakoff, J. A., Berman, R. F., and Ashwood, P. (2013). Maternal immune activation and strain specific interactions in the development of autism-like behaviors in mice. *Translational Psych.* **3**: e240.

Scott, B. L., and Bazan, N. G. (1989). Membrane docosahexaenoate is supplied to the developing brain and retina by the liver. *Proc. Nat. Acad. Sciences USA* **86**: 2903--2907.

Silvius, J. R. (1982). Thermotropic phase transitions of pure lipids in model membranes and their modifications by membrane proteins. **In** *Lipid--Protein Interactions*. John Wiley and Sons, Inc: New York, USA.

Smuts, C. M., Huang, M., Mundy, D., Plasse, T., Major, S., and Carlson, S. E. (2003). A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstetrics Gynecol.* **101(3)**: 469--479.

Sonuga-Barke, E. J. S., Brandeis, D., Cortese, S., Daley, D., Ferrin, M., Holtmann, M., Stevenson, J., Danckaerts, M., van der Oord, S., Döpfner, M., Dittmann, R. W., Simonoff, E., Zuddas, A., Banaschewski, T., Buitelaar, J., Coghill, D., Hollis, C., Konofal, E., Lecendreux, M., Wong, I. C. K., and Sergeant, J. (2013). Nonpharmacological interventions for ADHD: Systematic review and meta-analyses of randomized controlled trials of dietary and psychological treatments. *Am. J. Psych.* **170**: 275--289.

Srinivasarao, P., Narayanareddy, K., Vajreswari, A., Rupalatha, M., Prakash, P. S., and Rao, P. (1997). Influence of dietary fat on the activities of subcellular membrane-bound enzymes from different regions of the brain. *Neurochem. Int.* **31(6)**: 789-794.

Stevens, L. J., Zentall, S. S., Deck, J. L., Abate, M. L., Watkins, B. A., Lipp, S. R., and Burgess, J. R. (1995). Essential fatty acid metabolism in boys with attention-deficit hyperactivity disorder. *Am. J. Clin. Nutr.* **62(4)**: 761-768.

Stonehouse, W., Conlon, C. A., Podd, J., Hill, S. R., Minihane, A. M., Haskell, C., and Kennedy, D. (2013). DHA supplementation improved both memory and reaction time in healthy young adults: a randomized controlled trial. Am. J. Clin. Nutr. ***97****: 1134-1143.*

Storch, J., and Thumser, A. E. (2010). Tissue-specific functions in the fatty acid-binding protein family. J. Biol. Chem. ***285(43)****: 32679-32683.*

Stordy, B. J. (1999). 7 -- Docosahexaenoic acid: A dietary factor essential for individuals with dyslexia, attention deficit disorder and dyspraxia? ***In*** *Lipids in Health and Nutrition, Tyman, J. H. P. (ed.), pp. 102-114. Woodhead Publishing Limited: Cambridge, UK (*ISBN: 978-1-85573-790-7).

Sun, G. Y., Xu, J., Jensen, M. D., and Simonyi, A. (2004). PhospholipaseA2 in the central nervous system: implications for neurodegenerative diseases. *J. Lipid Res.* **45**: 205--213.

Surma, M. A., Klose, C., Klemm, R. W., Ejsing, C. S., and Simons, K. (2011). Generic sorting of raft lipids into secretory vesicles in yeast. *Traffic* **12(9)**: 1139-1147.

Taanila, A., Murray, G. K., Jokelainen, J., Isohanni, M., and Rantakallio, P. (2005). Infant developmental milestones: A 31-year follow-up. *Developm. Med. Child Neurol.* **47**: 581--586.

Takeuchi, T., Iwanaga, M., and Harada, E. (2003). Possible regulatory mechanism of DHA-induced anti-stress reaction in rats. *Brain Res.* **964**: 136--143.

Taoka, Y., Nagano, N., Okita, Y., Izumida, H., Sugimoto, S., and Hayashi, M. (2011). Effect of Tween 80 on the growth, lipid accumulation and fatty acid composition of Thraustochytrium aureum ATCC 34304. J. Biosci. Bioeng. ***111(4)****: 420-424.*

Tofail, F., Kabir, I., Hamadani, J. D., Chowdhury, F., Yesmin, S., Mehreen, F., and Huda, S. N. (2006). Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants. *J. Health Pop. Nutr.* **24(1)**: 48--56.

Tuohy, K., Venuti, P., Cuva, S., Furlanello, S., Gasperotti, M., Mancini, A., Ceppa, F., Cavalieri, D., de Filippo, C., Vrhovsek, U., Mena, P., Del Rio, D., and Fava, F. (2015). Chapter 15 -- Diet and the gut microbiota -- How the gut:brain axis impacts on autism. ***In*** *Diet-microbe interactions in the gut -- Effects on human health and disease (Tuohy, K., and Del Rio, D., eds.), pp. 225-245. Academic Press: London, UK (ISBN: 978-0-12-407825-3).*

Uddin, L. Q., Supekar, K., and Menon, V. (2010). Typical and atypical development of functional human brain networks: insights from resting-state fMRI. *Front. Systems Neurosci.* **4**: 21.

Van Elst, K., Bruining, H., Birtoli, B., Terreaux, C., Buitellar, J. K., and Kas, M. J. (2014). Food for thought: Dietary changes in essential fatty acid ratios and the increase in autism spectrum disorders. Neurosci. Biobehav. Rev. ***45****: 369-378.*

van Goor, S. A., Dijck-Brouwer, D. A., Erwich, J. J., Schaafsma, A., and Hadders-Algra, M. (2011). The influence of supplemental docosahexaenoic and arachidonic acids during pregnancy and lactation on neurodevelopment at eighteen months. *Prostaglandins Leukotrienes Essential Fatty Acids* **84(5-6)**: 139--46.

Vancassel, S., Durand, G., Barthelemy, C., Lejeune, B., Martineau, J., Guilloteau, D., Andrès, C., and Chalon, S. (2001). Plasma fatty acid levels in autistic children. *Prostaglandins Leukotrienes Essential Fatty Acids* **65**: 1--7.

Versantvoort, C. H. M., Oomen, A. G., Van de Kamp, E., Rompelberg, C. J. M., and Sips, A. J. A. M. (2005). Applicability of an *in vitro* digeston model in assessing the bioaccessibility of mycotoxins from food. *Food Chem. Toxicol.* **43**: 31-40.

Voigt, R. G., Llorente, A. M., Jensen, C. L., Fraley, J. K., Berretta, M. C., and Heird, W. C. (2001). A randomized, double-blind, placebo-controlled trial of docosahexaenoic acid supplementation in children with attention-deficit/hyperactivity disorder. J. Pediatrics ***139(2)****: 189-196.*

Voigt, R. G., Mellon, M. W., Katusic, S. K., Weaver, A. L., Matern, D., Mellon, B., Jensen, C. L., and Barbaresi, W. J. (2014). Dietary docosahexaenoic acid supplementation in children with autism. *J. Pediatric Gastroenterol. Nutr.* **58(6)**: 715-722.

Voss, A., Reinhart, M., Sankarappa, S., and Sprecher, H. (1991). The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *J. Biol. Chem.* **266(30)**: 19995--20000.

Weiser, M. J., Mucha, B., Denheyer, H., Atkinson, D., Schanz, N., Vassiliou, E., and Benno, R. H. (2016). Dietary docosahexaenoic acid alleviates autistic-like behaviors resulting from maternal immune activation in mice. Prostaglandins Leukotrienes Essential Fatty Acids **106**: 27-37.

Whelan, J. (2008). (n-6) and (n-3) Polyunsaturated fatty acids and the aging brain: Food for thought. J. Nutr. ***138(12)****: 2521-2522.*

Widenhorn-Müller, K., Schwanda, S., Scholz, E., Spitzer, M., and Bode, H. (2014). Effect of supplementation with long-chain □-3 polyunsaturated fatty acids on behavior and cognition in children with attention deficit/hyperactivity disorder (ADHD): A randomized placebo-controlled intervention trial. Prostaglandins Leukotrienes Essential Fatty Acids **91(1-2)**: 49-60.

Wiest, M. M., German, J. B., Harvey, D. J., Watkins, S. M., and Hertz-Picciotto, I. (2009). Plasma fatty acid profiles in autism: A case-control study. Prostaglandins Leukotrienes Essential Fatty Acids ***80****: 221-227.*

Willatts, P., Forsyth, S., Agostoni, C., Casaer, P., Riva, E., and Boehm, G. (2013). Effects of long-chain PUFA supplementation in infant formula on cognitive function in later childhood. *Am. J. Clin. Nutr.* 98: 536S-542S.

Won, H., Mah, W., and Kim, E. (2013). Autism spectrum disorder causes, mechanisms, and treatments: focus on neuronal synapses. *Front. Mol. Neurosci.* **6**: 19.

Yamada, K., and Nabeshima, T. (2003). Brain-derived neurotrophic factor/TrkB signaling in memory processes. *J. Pharmacol. Sciences* **91(4)**: 267--270.

Yang, L. Y., Kuksis, A., and Myher, J. J. (1990a). Lipolysis of menhaden oil triacylglycerols and the corresponding fatty acid alkyl esters by pancreatic lipase in *vitro*: a reexamination. *J. Lipid Res.* **31**: 137--147.

Yang, L. Y., Kuksis, A., and Myher, J. J. (1990b). Intestinal absorption of menhaden and rapeseed oils and their fatty acid methyl and ethyl esters in the rat. *Biochem. Cell Biol.* **68**: 480--491.

Youdim, K. A., Martin, A., and Joseph, J. A. (2000). Essential fatty acids and the brain: possible health implications. Int. J. Developm. Neurosci. ***18****: 383-399.*

Yui, K., Koshiba, M., Nakamura, S., and Kobayashi, Y. (2012). Effects of large doses of arachidonic acid added to docosahexaenoic acid on social impairment in individuals with autism spectrum disorders: a double-blind, placebo-controlled, randomized trial. *J. Clin. Psychopharmacol.* **32(2)**: 200--206.

Zeisel, S. H. (2009). Importance of methyl donors during reproduction. *Am. J. Clin. Nutr.* **89**: 673S--677S.

Zimmer, L., Vancassel, S., Cantagrel, S., Breton, P., Delamanche, S., Guilloteau, D., Durand, G., and Chalon, S. (2002). The dopamine mesocorticolimbic pathway is affected by deficiency in n-3 polyunsaturated fatty acids. *Am. J. Clin. Nutr.* **75(4)**: 662--667.

Zimmermann, M. B. (2011). The role of iodine in human growth and development. *Seminars Cell Developm. Biol.* **22**: 645--652.

Table 1 -- Overview of some significant intervention and observational studies concerning the effects of DHA on autism disorder, ADHD, and other behavioural disorders.

Type of Study	Study Length (months)	N*	Age (yrs)	DHA Intake (mg/day)	DHA Source/ Form	Outcome	Reference
Observational Study	---	26	3.05±0.79 (autistic) 3.87±1.06 (control)	---	---	Children with autism demonstrated lower red blood cell DHA levels than control children	Jory, 2016
Observational Study	---	80	<5	---	---	Autistic children had lower dietary intake of DHA as well as lower serum levels of DHA than controls	Al-Farsi et al., 2013
Observational Study	---	33	1-19	---	---	Marked reduction in DHA levels (23%) in the autistic subjects, without significant reduction in n-6 PUFA	Vancassel et al., 2001
Observational Study	---	160	4-12	---	---	Low plasma DHA was found in 67.5% of autistic children. High levels of anti-MBP antibodies correlated to low DHA	Mostafa et al., 2015
Observational Study	---	84	---	---	---	Patients with autism showed DHA levels in the polar lipids of red blood cells similar to control group	Bell et al., 2004
Observational study	---	250	2-5	---	---	DHA content in PE was lower in children (2-5 year old) with autism than in the control group	Wiest et al., 2009
Randomized Controlled Trial	3	60	3-11	240	Fish oil	After taking a supplement rich in DHA 66% of autistic children showed clinical and biochemical improvement	Meguid et al., 2008
Randomized Controlled Trial	4	13	14.6±5.9	240	---	Supplementation with high ARA+DHA doses improved social interaction in individuals with autism disorder	Yui et al., 2012
Randomized Controlled Trial	6	48	3-10	200	Algal oil	Dietary DHA supplementation of 200 mg/day for 6 months did not improve the core symptoms of autism	Voigt et al., 2014
Randomized Controlled Trial	6	38	2-5	---	---	This study did not support high dose supplementation of n-3 PUFAs in young children	Mankad et al., 2015
Observational study	---	89	9.1±2.3 (ADHD) 8.7±2.3 (control)	---	---	DHA levels were significantly lower in hyperactive children (41.6 □g/ml) than controls (49.5 □g/ml)	Mitchell et al., 1987
Observational study	---	23	10.4-16.6	---	---	ADHD children consumed as much n-3 and n-6 PUFA as controls, but they had lower DHA levels	Colter et al., 2008
Observational study	---	154	Mean age: 11.3	---	---	Long-term beneficial effects of DHA intake *in utero* upon behavioural performance assessments of memory	Boucher et al., 2011
Randomized Controlled Trial	4	362	7-9	600	Algal oil	DHA supplementation improved reading and behaviour in healthy but underperforming children	Richardson et al., 2012

Randomized Controlled Trial	4	53	8.91±1.73	1,032	Fish oil	Increasing erythrocyte DHA intake via dietary supplementation seemed to improve behaviour in ADHD children	Milte et al., 2015
Randomized Controlled Trial	4	95	6-12	120	---	DHA supplementation improved working memory function. No effect on parent-/teacher-rated behaviour	Widenhorn-Müller et al., 2014
Randomized Controlled Trial	3	166	9-12	514	Fish oil	It seemed to be possible that changes in FA nutrition, namely DHA, affected physical aggression especially in girls	Itomura et al., 2005
Randomized Controlled Trial	4	63	6-12	345	---	There was no improvement in any measure of ADHD symptoms after DHA supplementation during 4 months	Voigt et al., 2001
Randomized Controlled Trial	2	40	6-12	514	Fish oil	DHA supplementation did not improve ADHD-related symptoms	Hirayama et al., 2004
Randomized Controlled Trial	3	233	9-14	650	Fish oil	Behaviour checked with specific parameters did not show any difference between DHA and control groups	Hamazaki et al., 2008

*N -- Number of participants in the study.

Table 2 -- Average DHA content (mg/100 g) in different marine sources, not subjected to any culinary process (Bandarra et al., 2004; Makri et al., 2011; Nunes et al., 2006; Patil et al., 2007; Peinado et al., 2014; Taoka et al., 2011).

Category	Product	[DHA] (mg/100 g)	DHA Richness
Bivalves	Common cockle	215	Poor
	Grooved carpet shell	55	Poor
Cephalopods	Common cuttlefish	38	Poor
	Common octopus	129	Poor
	European squid	417	Medium
	Flying squid	225	Poor
Crustaceans	Norway lobster	77	Poor
	Red shrimp	28	Poor
	Rose shrimp	29	Poor
Fish	Alfonsino	48	Poor
	Atlantic cod	42	Poor
	Atlantic mackerel	1,580	Rich
	Atlantic salmon	1,773	Rich
	Auxillary seabream	327	Medium
	Black scabbardfish	171	Poor
	Blackspot seabream	490	Medium
	Chub mackerel	2,128	Rich
	Common sole	29	Poor
	European conger	425	Medium
	European eel	3,447	Rich
	European hake	155	Poor
	European plaice	153	Poor
	Gilthead seabream	1,207	Rich
	Greater forkbeard	26	Poor
	Horse mackerel	363	Medium
	Ling	21	Poor
	Meagre	147	Poor
	Monkfish	38	Poor

	Northern bluefin tuna	420	Medium
	Rainbow trout	387	Medium
	Red porgy	45	Poor
	Rubberlip grunt	79	Poor
	Sardine	1,169	Rich
	Sea bass	599	Rich
	Silver scabbardfish	460	Medium
	Smooth hound	51	Poor
	Swordfish	829	Rich
	Thornback ray	44	Poor
	Wreckfish	418	Medium
Microalgae	*Amphidinium sp.* S1*	677	Poor**
	*Isochrysis galbana NIVA-4/91**	1,580	Medium**
	Prorocentrum triestinum S2*	752	Poor**
	Thraustochytrium aureum ATCC 34304	6,590	Rich**
Seaweeds	*Ascophyllum nodosum**	40	Poor
	*Fucus spiralis**	83	Poor
	*Fucus vesiculosus**	91	Poor
	*Laminaria digitata**	16	Poor
	*Pelvetia canaliculata**	127	Poor

*For microalgae and seaweeds, DHA contents are given in mg/100 g of dry matter.

**For microalgae and seaweeds, richness was assessed assuming 20% dry matter as is usually the case in seafood.

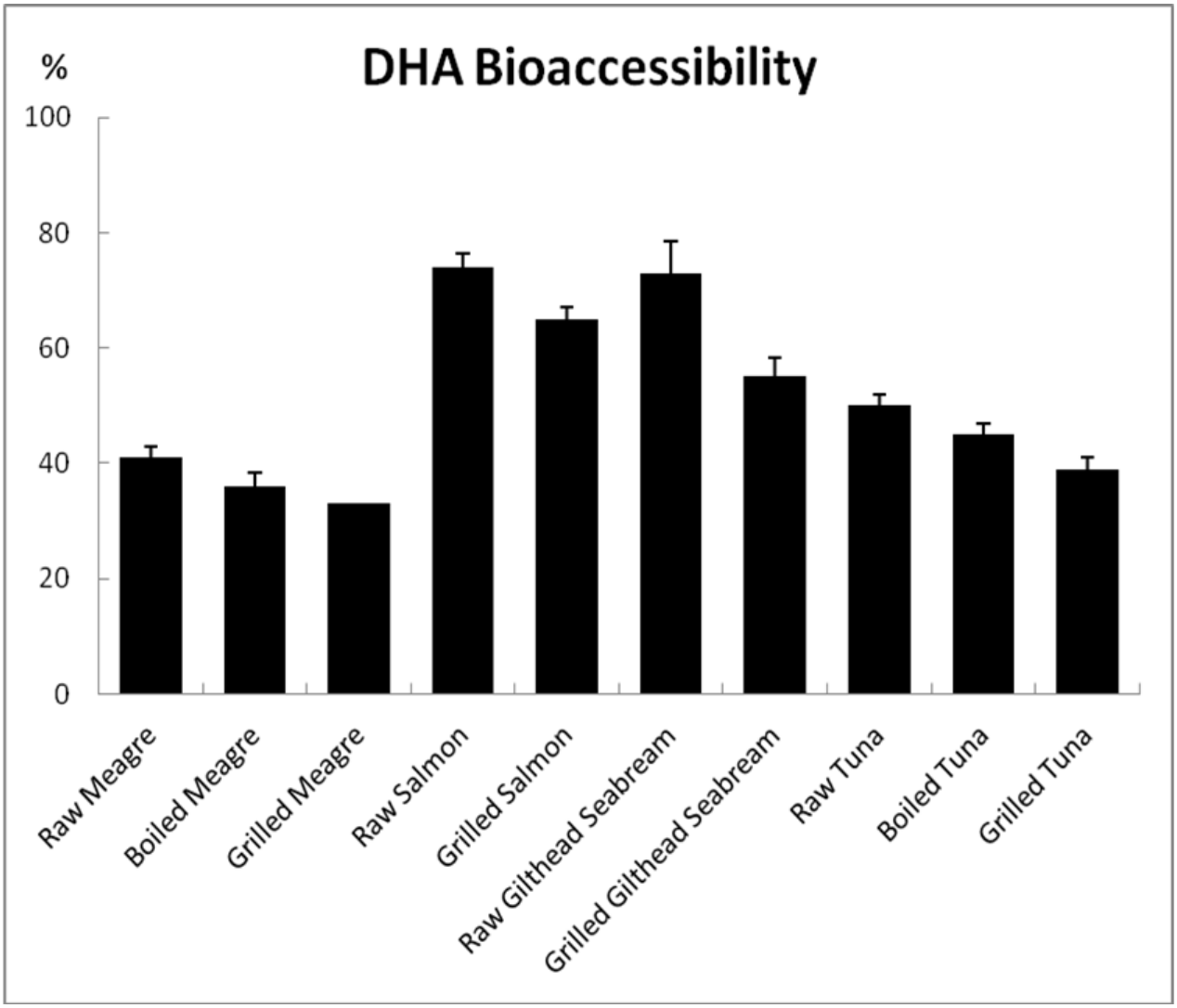

Figure 1 -- Dependence of DHA bioaccessibility on fish species and culinary treatment (Afonso et al., 2015; Bandarra et al., 2016; Costa et al., 2015, 2016).