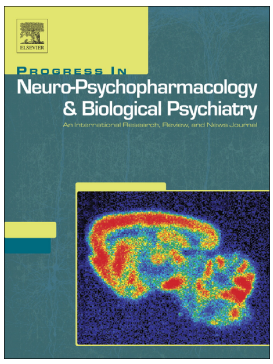
Accepted Manuscript



Omega-3 PUFA metabolism and brain modifications during aging

Laurie Chevalier, Hillary Chappus-McCendie, Claude Roberge, Mélanie Plourde

PII: S0278-5846(19)30097-1
DOI: https://doi.org/10.1016/j.pnpbp.2019.109662
Article Number: 109662
Reference: PNP 109662

To appear in: *Progress in Neuropsychopharmacology & Biological Psychiatry*

Received date: 4 February 2019
Accepted date: 28 May 2019

Please cite this article as: L. Chevalier, H. Chappus-McCendie, C. Roberge, et al., Omega-3 PUFA metabolism and brain modifications during aging, Progress in Neuropsychopharmacology & Biological Psychiatry, https://doi.org/10.1016/j.pnpbp.2019.109662

Review

Omega-3 PUFA metabolism and brain modifications during aging

Laurie Chevalier*, Hillary Chappus-McCendie*, Claude Roberge and Mélanie Plourde[1,#]

[1] Research Center on Aging, Health and Social Services Centre – University Institute of Geriatrics of Sherbrooke, Department of medicine, Université de Sherbrooke, 1036 Belvédère Sud, Sherbrooke, Canada, J1H 4C4; E-Mails: Melanie.plourde2@usherbrooke.ca (M.P);

*LC and HCM equally contributed to the redaction and the revision of the manuscript and are considered co-first authors.

#Author to whom correspondence should be addressed;

E-Mail: Melanie.plourde2@usherbrooke.ca

Phone number: +1-819-780-2220 extension 45664;

Fax: +1-819-829-7141.

Abstract:

In Canada, 5.5 million (16% of Canadians) adults are >65 years old and projections suggest this number will be approximately 20% of Canadians by 2024. A major concern regarding old age is a decline in health, especially if this entails a loss of self-sufficiency and independence caused by a decline in cognition. The brain contains 60% of fat and is one of the most concentrated organs in long chain omega-3 fatty acids such as docosahexaenoic acid (DHA). During aging, there are physiological modifications in the metabolism of lipids that could also have consequences on brain structure and levels of DHA. This review will hence discuss the physiological modifications in the metabolism of lipids during aging with a focus on long chain omega-3 and omega-6 fatty acids and also outline the structural and functional modifications of the brain during aging including brain lipid modifications and its relation to higher levels of DHA and cognition. Therefore, in this review, we outline the importance of collecting more data on the biology of aging since it might highly improve our understanding about what are «normal» modifications occurring during aging and what can become pathological.

Keywords: lipid metabolism, aging, docosahexaenoic acid, fatty acids, brain structure, brain function,

1. Introduction

Almost every country in the world experiences an aging population, and this population is expected to be one of the most significant forces shaping our economy and society in the next 20-30 years. A major concern about old age, both at the individual and societal levels, is a decline in health, especially if this means a loss of self-sufficiency and independence. Increasing research aimed at promoting healthy aging is actually ongoing but one of the major hurdles is to define the biology of aging. Aging in humans refers to a multidimensional process of physical, psychological, and social changes. Therefore, it follows that fundamental knowledge on the biological processes occurring during aging may help to design environmental strategies aimed at promoting healthy biological aging. Thus, there is a need for better prevention strategies, but one major gap in this field is a need to better understand what the biological modifications are, also called geroscience, since this field is relatively new. One of the strategies to promote healthy aging is the consumption of one or two fish meals each week [1-3]. Normally, the intake of fish positively correlates with increased plasma and erythrocyte omega-3 fatty acids (n-3 FAs), likely with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in a time- and dose-dependent manner [4-6]. EPA and DHA have to be provided through the diet because their synthesis from their precursor alpha-linolenic acid (ALA) is extremely limited in humans [7]. However, over the 20th century, the dietary fat consumption has drastically changed with an increased level of omega-6 fatty acids (n-6 FAs) such as linoleic acid (LA) from 2.79% to 7.21% of energy. This shift in our dietary fat intake was largely due to our dependence on new food production methodologies, including soybean oil [8].

The link between our dietary fat intake and the incidence of chronic diseases has been largely debated over the last 20 years. Our research group is mainly focused on prevention of cognitive decline, so the focus of this review paper, with respect to chronic diseases, will be on cognition. This link between dietary fat intake and the risk of cognitive decline has been the focus of many review papers overs the last 10-15 years [9-11]. One of the most recent reviews supports a positive association between dietary and blood n-6: n-3 ratio and cognitive decline and incidence of dementia, as evaluated on 14 human studies including 7 prospective studies [12]. A recent meta-analysis on 11 cohort studies evaluated the association between 299 metabolites and general cognitive ability and dementia. They reported that higher DHA levels in blood were associated with higher cognitive function in 22,887 individuals [13]. Hence, it seems that a more elevated concentration of n-3 FA in the blood is associated with lower cognitive decline and perhaps a lower risk of other chronic diseases. However, our group showed that for older participants, plasma EPA and DHA kinetics are dysregulated and this will likely lower the capacity of older adults to

incorporate EPA and DHA in organs and tissues [14, 15]. Usually, a fish oil supplementation increases the level of EPA and DHA in the plasma or erythrocytes but in those aged >70 years old, we don't know whether this process is efficient. There is no clear definition or parameters to define an old vs. a young participant. Most of the studies used the median of age in their participants group or a continuous age range. Following from the information summarized above, this paper will review some of the metabolism modifications occurring during aging with a focus on lipid metabolism. By reviewing these evidences, we will also expose how these modifications might limit incorporations of n-3 FA in membranes of cells with a focus on the brain because it is one of the most enriched organs in DHA.

2. Lipid and fatty acid metabolism differences during aging

Generally speaking, there are differences in the lipid and fatty acid metabolism occurring during aging and these modifications are considered totally normal and part of the aging process. These processes include the transport of fatty acids after their intake and their transit to the different organs and tissues that are modified during aging. This section will review some of these modifications.

2.1.Normal transport of fatty acids from dietary intake to their circulation in the blood:

In Western adults, the diet is composed of 30 to 40% of lipids, of which 92 to 96% are long chain fatty acid esterified to a glycerol thus constituting what is the main form of dietary lipid: triglycerides (TG) [16]. Whole-body homeostasis requires fine-tuning of fatty acid transport and utilization by metabolically active tissues [17]. Because of their regulatory roles in cellular fatty acid uptake and utilization, membrane apolipoprotein receptors and fatty acid transporters form an integral part of this homeostatic system. As a result, imbalances in lipid metabolism will likely influence the functioning of fatty acid transporters and their protein levels. Lipids are not soluble in water and necessitate incorporation into amphiphilic molecules called lipoproteins to circulate in the blood. Hence, following ingestion of TG, they will be hydrolysed at their ester bonds by gastric and pancreatic lipases into two non-esterified fatty acids (NEFA) and one monoacylglycerol (MAG) with the fatty acid being in the *Sn*-2 position [18]. Both forms of lipids are passively transported into enterocytes [19] via diffusion or transporters such as "Fatty Acid Transport Proteins" (FATPs) and "Cluster of Differentiation 36" (CD36) [20]. Dietary lipids are efficiently digested and absorbed by the enterocytes [21]. Once inside the intestinal cells, enzymes convert the NEFAs and MAG back into TG [22]. These will be integrated in chylomicrons and exported to the lymphatic system via the Golgi apparatus [23]. The chylomicrons, now rich in exogenous TGs, join the bloodstream via the thoracic duct and get transported to the peripheral tissues such as muscle and fat cells. In the

bloodstream, lipoprotein lipase (LPL) gets activated when it detects an apolipoprotein C II (apoC-II) [24] on the surface of the chylomicrons. The role of LPL is to hydrolyse the ester bonds of TGs in chylomicrons [24] to release NEFAs into the bloodstream where there will be an uptake by nearby cells. The loss of TGs will result in a decrease in size of chylomicrons and leave chylomicron constituents available for the synthesis of native HDL disks [25]. Remnant chylomicrons rich in cholesteryl esters will be captured by endocytosis by hepatocyte receptors such as LDL receptor (LDLR) [24] and LDL receptor-related protein (LRP) [26]. The liver can then use the endogenous TG and cholesteryl esters to form very low density lipoprotein (VLDL) [27]. These lipoproteins will be directed to peripheral tissues. Following a loss of TG, there will be a decrease in VLDL density [28]. With the action of LPL, VLDL will then become intermediate density lipoprotein (IDL). With the action of hepatic lipase [28] IDL becomes low density lipoprotein (LDL). LDLs carry cholesterol to tissues [29]. LDL will be captured by their receptor, LDLR which are found on cell membranes, where it will be eliminated from the bloodstream by endocytosis [29]. LDL cholesterol will be recovered in the cell. An excess of cholesterol in the tissues will cause an inhibition of transcription of the genes responsible for the formation of the LDLR [30]. It thus reduces the uptake of LDL by the cells and these LDLs will remain in circulation. The remaining LDLs in the circulation are more likely to be oxidized [31] which will thereafter contribute to the development of atherosclerotic plaque [31].

2.2 Lipoprotein metabolism modification during aging

During aging, the metabolism of lipids is modified and causes an increase of plasma lipids. For instance, the fasting plasma levels of VLDL, TG, LDL and cholesterol [32] are significantly higher in the elderly [33]. Higher levels of lipids and cholesterol can be the source of many health problems such as cardiovascular disease (CVD) and diabetes [34, 35].

These plasma lipid changes in the elderly can cause an increase in plasma free fatty acid levels [36]. Increasing plasma FFA may result in increased plasma glucose by decreasing glucose uptake into the cells. The enzymes responsible for the oxidative cascade of gamma-linolenic acid are intimately related to that of glycolysis. Thus, increased lipid oxidation inhibits glucose metabolism, decreases glucose uptake in cells, and impairs glycogen storage [37]. This promotes hyperinsulinemia and ultimately insulin resistance [36].

Insulin resistance, often seen in the elderly [38], will also cause an increase in VLDL and blood TGs. It also impairs the metabolism of chylomicrons, VLDL, LDL and HDL [39] since a lack of insulin or a lower sensitivity to insulin will reduce the catabolism of chylomicrons and VLDL by LPL. During aging, there is also a higher level of LDL which remains transient for a longer period of time in the plasma [33]. In the long term, these LDLs are more likely to be oxidized [39]. The higher concentration of VLDL and chylomicrons in addition to oxidized LDL accumulation in older insulin-resistant individuals would increase the risk of CVD [40]. Furthermore, the increase of LDL may be due to the diminution of bile synthesis from cholesterol by the liver during aging [33, 41]. The decrease in bile acid synthesis is due to the decrease in the expression of "cholesterol 7-alpha hydroxylase" (CYP7A1) during aging. This cytochrome is one of the CYP450 and regulates the formation of bile acids [42]. This causes a decrease in the use of cholesterol by the liver as well as a reduction in LDLR expression with age. Thus, plasma LDL will have lower clearance with age resulting in an increase in plasma LDL concentration in the elderly [32]. In the end, it is possible that deregulation of LDL in the elderly is due to several different phenomena stemming from the large amount of change that occurs with age. The decrease in LDL in the elderly has shown a reduction in the incidence of CVD [43]. In particular, a study showed that long chain polyunsaturated fatty acids (PUFAs) allowed an increase in LDLR expression [44], which could increase the clearance rate of plasma LDL in the elderly and reduce the incidence of CVD. These are some of the modification of the lipid metabolism occurring during aging. Overall, there are usually higher TG and LDL levels in the blood of older adults and it is important to consider these modifications in the prevention of chronic diseases but also when interpreting results pertaining to fatty acid metabolism.

2.3 N-3 FA metabolism during aging

Over the last 10 years, our group has worked on n-3 FA metabolism with a focus on modifications that occur during aging. This section will report the evidence of n-3 FA metabolism in three different conditions: before supplementation with n-3 FAs, during or after supplementation with n-3 FAs, and kinetics studies using uniformly labeled carbon 13 fatty acids (^{13}C-).

2.3.1 Without an n-3 FA supplementation

To our knowledge, there are ~ 23 studies that have reported the level of n-3 FAs or the n-3 FA index in young versus old adults (Table 1). Most of the studies reported the fatty acid profile in red blood cells or in plasma/serum phospholipids (PL). Red blood cells has a more complete spectrum of

phospholipid classes and therefore a FA composition that may better reflect cell membranes of biological tissues [45, 46] whereas fatty acid profile in the plasma is more representative of the fatty acid circulating in the lipoproteins. Among the 23 studies, seven studies reported the n-3 FA index only and showed that it was higher in older participants [47-53]. Three studies on the n-3 FA index reported an increase of about 5-7% of the n-3 FA index every decade [47, 50, 51]. Twelve studies reported the fatty acid profile in red blood cells (RBC) [47, 48, 51-60]. For most of the studies, it is difficult to compare the results since the data were not expressed the same way. For instance, two studies reported that the participants having the highest level of n-3 FAs were on average 8-10 years older than those with the lowest n-3 FA levels in erythrocytes [53, 54]. Other studies reported the level of increase in n-3 FAs for each increasing decade. Hence, it is difficult to draw a clear conclusion for the n-3 FA results in RBC but it appears that at older ages, there is more n-3 FA in RBC which might reflect modifications of the fatty acid profiles in organs and tissues during aging. It is important to note that these papers did not include a complete fatty acid profile of the RBC as it was recently recommended in a paper describing the best practices for the design, laboratory analysis and reporting of clinical trials involving fatty acids [61], hence limiting comparisons between studies. Moreover, there are eleven studies that reported the fatty acid profile in plasma or serum [60, 62-71]. Seven of the studies reported on average a 35% higher level of DHA in the plasma/serum of older versus younger participants [60, 63, 65-67, 69, 71]. Similarly, eight of the studies reported on average a 88% higher level of EPA in the plasma/serum of older versus younger participants [60, 62, 63, 65-67, 69, 70]. One study reported a 114% higher level of EPA in the plasma PL of older participants but no difference between age groups for DHA [62]. Other studies reported ~~only~~ a positive correlation between age and EPA+DHA in plasma PL but it was not possible to quantify the magnitude of the difference between young and older adults [64, 68]. Overall, there is generally good evidence supporting the idea that during aging, the relative % of n-3 FAs or their concentration in RBCs and plasma/serum are higher in the oldest participants compared to the youngest. These studies did not offer a n-3 FA supplement. Some of the proposed mechanism includes a reduction of n-3 FAs in cell membranes, higher intestinal absorption during aging, higher availability and release of adipose tissue stocks. Hence, the exact mechanism behind this higher level of blood n-3 FAs in older individuals might be multi-level, but the important point here is that they might be associated to longevity.

2.3.2 With an n-3 FA supplementation

To our knowledge, there are nine published studies specifically addressing EPA and DHA responses to an n-3 FA supplement with participants of different ages (Table 2). Supplementation doses range from 300 mg/d to more than 4 g/d and lasted between 6 weeks and twelve months. Seven studies evaluated the fatty acid profile in the plasma [72-78] whereas one study evaluated the fatty acid profile in erythrocytes only [79] and another did so in platelets and adipose tissues only [80]. One study reported the n-3 FA index in RBCs pre- and post-supplementation [79] and showed that a low n-3 FA index at baseline and an older age predicted those with a greater increase of the n-3 FA index after supplementation [79]. This study had similar results to Vandal et al. [77], which showed that the oldest participants had a higher increase in DHA compared to the youngest participants after supplementation. However, DHA levels were similar in young and old participants at baseline in the Vandal study.

The other studies investigated the plasma level of n-3 FAs. One study reported that older participants had higher n-3 FA levels at baseline but after the supplementation, the increase was similar in both groups [72]. The six other studies reported a higher increase in EPA [73-76, 78] and/or DHA [77] in older participants compared to younger. The exact mechanism explaining this effect is unclear. Most of the studies reported that it is unlikely that the age-related differences in EPA and DHA at baseline are due to differences in intake of n-3 PUFAs with age. Rather it seems to be related to age differences in endogenous production and incorporation of EPA and DHA due to hormones and hormone sensitivity, body composition, and physical activity, all of which change with age [78]. The study of Walker et al. also showed that the adipose tissue stores less DHA with age in response to EPA + DHA supplementation, hence suggesting that age-related differences in the handling and storage of exogenous supplied DHA may be related to impaired insulin sensitivity with aging or to differences in body composition with aging [78]. The adipose tissue represents a significant store of EPA and DHA, containing the equivalent of several hundred days of the fatty acid content of a typical diet. Altogether, these results support that providing a supplement of n-3 FAs to older adults increases their blood levels when compared to younger individuals. These results may be caused by the fact that older individuals have shown to be more compliant to treatments than younger people [81], causing a higher level of omega-3 in their blood. But despite that fact, those results bring into question whether this type of supplementation is useful to older individuals in the prevention of chronic diseases since they may not be able to use it. Another important point is that it might also be due to their lower turnover of circulating TG ~~and LDL~~, hence contributing to their higher n-3 FA levels, since n-3 FA levels are esterified in TG ~~and bound to LDL~~. To answer some of these questions, employing ^{13}C-fatty acids is useful.

2.3.3 Using ^{13}C-fatty acid to evaluate their kinetics during aging

Tracing metabolism of ^{13}C-fatty acids may provide some insight into possible age-related changes in fatty acid metabolism in humans. Metabolism of ^{13}C-DHA has been investigated in humans [82-84]. In young adults given an oral dose of 250-280 mg ^{13}C-DHA, ^{13}C enrichment peaked at 2 h post-dose in plasma triglycerides when the tracer was given in the triglyceride form, but at 6 h post-dose when the tracer was esterified to phosphatidylcholine [82, 84]. Brossard *et al.* have reported a 1.4% apparent retro-conversion of ^{13}C-DHA to ^{13}C-docosapentaenoate (22:5 omega-3) and ^{13}C-EPA 3 d after giving the tracer [83]. These first results showed the feasibility of tracing DHA metabolism in humans. However, neither the impact of aging on ^{13}C-DHA metabolism nor its β-oxidation were investigated, although both may influence the somewhat higher blood levels of EPA and DHA commonly seen in healthy elderly [64, 75, 76, 85, 86]. Our group are pioneers in this field as we investigated the kinetics of ^{13}C-DHA in six young and six elderly participants [15]. We found that, in the elderly, ^{13}C-DHA was 4 times higher in plasma triglycerides and NEFA at 4 h post-dose, β-oxidation was 1.9 times higher, whereas apparent retro-conversion of ^{13}C-DHA to other ^{13}C-n-3 FAs was 2.1 times higher 24 h and 7 d after tracer intake compared to the young adults [15]. Hence, because DHA seems to remain transiently for longer periods of time in the blood of the elderly compared to the young, it may thus indicate that efficiency to remove DHA from the blood is lower in the elderly than in the young, resulting in lower incorporation of DHA in the membrane of cells that serve to initiate signalization [75, 76, 85, 86]. This result is consistent with the transient slower metabolism of TG and LDL in older as compared to young adults and this was described in a previous section.

Our most recent work with tracers between old and young men was conducted with ^{13}C-EPA or arachidonic acid (^{13}C-ARA), two key fatty acids that are precursors of anti- and pro-inflammatory cytokines, respectively. Surprisingly, the kinetics of ^{13}C-EPA and ^{13}C-ARA was quite similar between young and old men despite a time x age interaction for ^{13}C-EPA kinetics where the postprandial shape of the curve was steeper in old vs young men [14]. One intriguing result we obtained was that in old men, synthesis of DHA from EPA started 2 h after tracer intake whereas it was delayed to 1 d in young men. This result suggests that old adults might need more DHA than what was actually provided in their diet compared to the young men. However, newly synthesized DHA accumulated in the plasma of old men for 7 d and this might be because it remains for a longer period in the plasma as suggested by our previous study with ^{13}C-DHA. Therefore, there might be a

defect in old adults to uptake DHA in the tissues. We also calculated that plasma half-life of ^{13}C-EPA was 2 d whereas that of ^{13}C-ARA was 4 d, similar to that of DHA. DHA and ARA are the two most concentrated long chain polyunsaturated fatty acids in brain membranes. With our β-oxidation measures using breath samples, we calculated ^{13}C-EPA whole-body half-life to be ~14 days in old men whereas in the younger group it was ~21 days [14]. This result indicates that older adults turn over EPA ~7 days faster than the younger adults. This is an intriguing result since epidemiological studies and results we obtained in previous studies [72, 75] support that old adults have twice as much plasma EPA, hence one would anticipate a lower whole-body turnover in old vs young adults. Therefore, it seems that there is somehow a disconnect between plasma levels of EPA and perhaps DHA and their kinetics, thus more studies are needed to understand the mechanism of these modifications and their possible consequences such as potential higher risk of cognitive decline.

3 Brain modifications during aging:

The brain is composed of 60% fat with one third of its content being ARA and DHA. The brain is therefore the second most rich tissue in fat after adipose tissue. The brain fatty acids are however mostly PLs unlike the adipose tissue that is mainly composed of TGs. Because DHA is an important constituent of brain structure, there has been much interest in the association between the level of DHA in brain membranes, brain function and brain volume, and losses during aging. Therefore, this section will summarize the evidence about morphological, functional, and content modifications of the brain during aging and whether dietary n-3 FA intake can improve brain structure and function.

3.1. Morphological modifications of the brain during aging

There are a number of morphological modifications of the brain that occur during aging. Several studies have indicated that brain volume decreases over the course of the human lifespan. A review conducted by Hedman et al. [87] compiled the results of 56 longitudinal magnetic resonance imaging (MRI) studies on whole brain volumes in healthy individuals and concluded that the rate of total brain volume loss is not constant throughout aging. For instance, the rate of brain volume loss after 35 years of age is approximately 0.2% per year. Between 35 and 60 years of age, the volume loss rate slowly increases to 0.5% followed by a steady volume loss of over 0.5% per year over 60 years of age [87]. Furthermore, other studies have indicated that volume loss in the whole brain is greater in males than in females [88, 89].

Several studies demonstrate a reduction of grey matter volume during aging [90-96]. More specifically, the volume of grey matter in the cortex and the cerebellum of older individuals is 18% and 13% smaller, respectively, than those of their younger counterparts [93]. There is also a significant loss of grey matter in the frontal, limbic, temporal, and parietal lobes but not in the occipital lobe [90, 95]. Similarly, studies have also indicated that there is a decrease of white matter volume in the brains of older individuals [93, 97-99]. According to Jäncke et al. [93], there is a decrease in white matter volume in the cortex and cerebellum of older individuals by 5% and ~9%, respectively, compared to younger adults. Moreover, one study indicated that the rate of decrease of white matter volume is not constant during aging [99]. Instead, white matter volume slowly increases before the age of 40, peaks at approximately 50 years of age, and then quickly decreases after the age of 60 [99]. As well, white matter hyperintensity lesions increase in size with age in the frontal, temporal, and parietal lobes but not in the occipital lobes [98].

In addition to age-related changes in the volume of the whole brain, grey matter, and white matter, there are also differences in the volume of specific brain structures. There seems to be a general decrease in the volume of the following brain structures in older individuals compared to younger individuals: cerebral hemisphere [88], frontal lobe [100, 101], parietal lobe [89, 100, 101], temporal lobe [100, 101], thalamus [93, 102], basal ganglia [101], and the cerebellum [101]. Notably, there is atrophy of the hippocampus during aging [89, 93, 103-105]. A meta-analysis by Fraser et al. [105] detailed hippocampal atrophy rates according to 28 studies. They determined that the overall rate of atrophy for the entire sample was 0.85% per year [105]. However, the rate of hippocampal atrophy reported in the studies differed based on mean age of the participants: rate of atrophy was 0.38% per year in studies with a mean age of 55, 0.98% per year for a mean age of 55 to 70 years, and 1.12% per year for a mean age of greater than 70 years. In contrast to the aforementioned structures, the ventricles of the brain increase in volume during aging [88, 103]. Altogether, there is generally good evidence supporting loss of matter in many brain structures, including loss in white and grey matter. These losses of brain matter can contribute to lower cognitive functions during aging.

3.2 Modification of brain functions and cognition during aging

In addition to the many structural changes that occur during aging, brain functions are also modified during this period. For instance, there is an age-related decrease in glucose metabolism in the whole brain and the frontal, parietal, and temporal lobes as well as in Broca's and Wernicke's areas [89]. It also seems that brain activation during the execution of motor functions is modified in older adults. For example,

there is a decrease in blood-oxygen level dependent (BOLD) signals in multiple brain regions (sensorimotor cortex, cerebellum and thalamus) of older adults during mastication and an increase in BOLD signal in the prefrontal area [106]. Another study showed that classical motor coordination regions were activated during complex inter-limb coordination tasks, but that there was also increased activation of higher-level sensorimotor and frontal regions in older individuals [107]. Similarly, other studies have demonstrated that the performance of motor tasks result in increased activation of additional brain areas such as the basal ganglia, prefrontal cortex, precuneus, and the cerebellum [108-110] in older individuals.

Moreover, cognitive functions are modified as a result of changes in the volume of various brain structures. For instance, a meta-analysis of 57 publications from the years 1984 to 1998 concluded that white matter hyperintensities are linked with poorer performance on cognitive tests for processing speed, immediate and delayed memory, executive functions, and global cognitive functioning [111]. Further, a decrease in the thalamus volume in older individuals is associated with attenuated performance on tests assessing cognitive speed [102]. An additional meta-analysis of 33 studies concluded that larger prefrontal cortex volume and thickness is correlated with better executive functioning [112]. In regard to hippocampus volume and memory, Van Petten [113] reported in a meta-analysis of 33 studies that the positive correlation between hippocampus size and episodic memory in older adults was weaker than expected. However, a more recent study demonstrated that smaller hippocampus size is significantly associated with lower performance in episodic memory, working memory, processing speed, and executive function tasks [114]. Similarly to motor function, it has been shown that older adults recruit additional brain regions during memory tasks [115-117].

Aging is also associated with changes in the activity of brain structures involved in sensation and perception. For instance, there are less areas activated in older versus younger adults in response to various odors [118]. A meta-analysis of 105 studies concluded that the activation of the fusiform gyrus, cerebellum, and hippocampus is elevated in elderly versus younger individuals during the processing of emotional faces [119]. Moreover, older individuals had greater activation of the prefrontal cortex during more difficult perceptual tasks compared to younger individuals [120]. The brains of older adults are also less responsive to blue light stimulation compared to younger adults [121].

More recent studies have shed light on the changes that occur in the functional neural networks of the brain. It seems that aging is associated with weaker connectivity in long-range connections and stronger connectivity of short-range connections [122, 123]. Elderly individuals also have less intra-network and greater inter-network connectivity [124, 125]. More specifically, older individuals have less connectivity

within the default mode network (DMN) and somatomotor network [125], as well as greater connectivity between the salience network and the executive control network (ECN) and the DMN [124]. Moreover, age seems to shift dynamic functional connectivity from posterior to anterior regions, which is also reflected in the decreased activation of posterior regions during the decline of episodic memory in older individuals [126].

Overall, there are several morphological and functional modifications within the brain during aging and understanding how these modifications manifest could be helpful to limit the rate at which these declines occur.

3.3 Modifications of brain content during aging

The number of studies, particularly those that use neuroimaging techniques, that have evaluated the change in human brain content during aging is limited. Post-mortem examinations of the human brain have indicated that there is a change in protein and lipid content during aging. With regard to protein, one study indicated that there is a 5-15% decrease in total protein content of the brain between 30 and 90 years of age [127]. A decrease in protein content in the substantia nigra, hippocampus, caudate nucleus, and grey matter has also been reported [128, 129]. However, Söderberg et al. [128] found that protein content remained unchanged in the cerebellum, pons, and medulla oblongata of older individuals. Similarly, a number of post-mortem studies have demonstrated changes in the lipid content of older brains. For instance, Svenerholm et al. [130] reported that there is a linear decrease in cholesterol and phospholipids in the frontal and temporal cortices and a curvilinear decrease in cholesterol, PLs, cerebrosides, and sulfatides in frontal and temporal white matter between the ages of 20 and 100. In terms of PLs, Söderberg et al. [128] found that they were relatively unchanged during aging with only a 5-10% decrease in the oldest age group. A more recent study conducted by Hancock et al. [131] reported that PL content in the entorhinal cortex of older individuals is relatively stable during aging, but there is an increase in mitochondrial phosphatidylcholine (PC) and a decrease in mitochondrial phosphatidylethanolamine (PE). The same group reported that age is associated with an increase in mitochondrial PE containing DHA, but said the increase is not large enough to increase total DHA in the mitochondria. Norris et al. [132] examined phospholipid composition in the dorsolateral prefrontal cortex in individuals aged 20-100 years. They found that there is a general age-related increase in phospholipids containing DHA and decrease in PLs containing ARA and docosatetraenoic acid [132].

A recent study used positron emission tomography to assess the incorporation of DHA from plasma to the brain using carbon-11 ([1-C^{11}])-DHA in apolipoprotein E epsilon 4 allele (APOE4) carriers versus non-carriers [133]. APOE4 is the most important genetic risk of late-onset Alzheimer's disease [134]. Yassine et al. found that the mean global grey matter DHA incorporation coefficient was 16% higher in APOE4 carriers vs non-carriers [133]. A higher DHA incorporation coefficient was also observed in other regions including the entorhinal cortex [133]. However, the whole-brain DHA incorporation rate was not significantly different between APOE groups [133]. They also did not observe any age-related effects on DHA incorporation, but this may be due to the fact that only 4 of their 23 participants were over 50 years old [133]. The authors hypothesized that increased DHA incorporation in the brains of APOE4 carriers could be a compensatory mechanism to counteract brain DHA loss [133]. Our group also documented that the metabolism of DHA is imbalanced in APOE4 carriers [135-138] and that they are perhaps more vulnerable to DHA deficiency [139].

3.4 Does n-3 FA consumption improve brain structure and function?

There are a number of studies that have examined the relationship between n-3 FA consumption and brain structure and function. For instance, Gu et al. [140] evaluated the link between white matter integrity and dietary nutrient intake in 239 elderly participants. They assessed white matter integrity using fractional anisotropy measured by diffusion tensor imaging (DTI). They found that the nutrient pattern characterized by high consumption of n-3 and n-6 FAs and vitamin E was positively correlated with fractional anisotropy which corresponds to better white matter integrity [140].

Another group examined the relationship between dietary fish consumption and brain structural integrity in 260 cognitively normal adults aged 65 years or older [141]. Fish intake was measured using the National Cancer Institute Food Frequency Questionnaire and the grey matter volume of various brain regions was measured with MRI [141]. They found that eating baked or broiled fish weekly is positively associated with higher grey matter volume in several brain regions, including the hippocampus, posterior cingulate, precuneus, and the orbital frontal cortex [141].

Samieri and colleagues [142] evaluated the association between plasma EPA and DHA concentrations and grey matter atrophy in the medial temporal lobe in 281 individuals aged 65 years or older. The authors compared fatty acid plasma concentrations at baseline to the results of MRI examinations from baseline and four years after baseline [142]. They observed that greater levels of plasma EPA was associated with lower atrophy of the grey matter of the right amygdala and the hippocampal/parahippocampal region; this

same association was not observed for plasma DHA levels [142], which is counterintuitive. Samieri et al. [142] also found that increased amygdala grey matter atrophy was linked with more depressive symptoms and poorer semantic memory performances compared to baseline.

Lastly, Witte et al. [143] assessed the connection between fish oil supplement consumption and brain structure and function in 65 participants aged 50 to 75 years. Participants consumed either fish oil, which contained 2.2 grams of n-3 FAs, or a placebo daily for 26 weeks. Neuropsychological testing and MRI examinations were performed before and after the intervention period. The investigators found that after the 26-week intervention period, the fish oil group had better white matter structural integrity in selective white matter tracts in the frontal, temporal, parietal, and limbic areas [143]. They also observed that the fish oil group had significant increases in grey matter volume in the left hippocampus, precuneus, the superior temporal, inferior parietal and postcentral gyri, and in the right middle temporal gyrus [143]. In terms of performance on cognitive measures, they found that the fish oil group had an improvement of 26% on executive function scores compared to no improvement in the placebo group [143]. In addition, they found a positive correlation between verbal fluency scores and EPA percentage in red blood cell membranes in the fish oil group after intervention [143]. While the aforementioned studies suggest that n-3 FA consumption in older individuals may improve brain structure and cognition, the mechanism behind this beneficial effect is not yet known and must be further investigated.

Although for many years it was thought that an intake of fish throughout life protects against cognitive decline, the recent evidence suggests that fish intake might not be required throughout life to improve brain structure and function. Hence, starting an EPA+DHA supplementation after 50 years old might benefit older individuals with respect to prevention of brain volume and function losses.

4 **Are we ready for updated recommendations on dietary n-3 FAs intake during aging?** In this review paper, we have outlined that there are many physiological modifications occurring during aging with respect to lipid metabolism and brain volume and function losses and that an n-3 FA intake might help to support the brain throughout aging. It is important to note that life expectancy is longer, which means that older adults may live longer with their chronic diseases. A major concern regarding old age is a decline in health, especially if this entails a loss of self-sufficiency and independence caused by a decline in cognition. A decline in working memory appears to be one of the major consequences of *normal* aging [144, 145]. As outlined in the previous sections, the brain undergoes physiological

change during aging. While age is one risk of cognitive decline, this multifactorial disease is also increased by a complex interaction between both genetic and environmental risk factors [146-148].

We believe nutrition has a role to play in the prevention of cognitive decline but nutrition alone might not be as efficient as a multidomain intervention. Recent evidence from the FINGER trials [149] reported that combining physical exercise, personalized nutritional recommendations to avoid nutrient deficiencies, controlling cardiovascular risks and having cognitive stimulation prevented cognitive decline. However, they recently refocused their message by showing that dietary changes initiated early in the intervention were the most influential for global cognition improvement over two years of follow-up [150]. Therefore, nutrition might have a key role to play in the prevention of cognitive decline. In the case of the FINGER study, dietary recommendations were not focused on the consumption of fish oil but were instead focused to alleviate nutritional deficiency including low blood levels of DHA. It also has to be emphasized that there is currently no drug to prevent, cure or delay the progression of dementia and that some pharmaceutical companies have shut down their research laboratories in this area. Therefore, prevention strategies are currently the most efficient means since once the disease process has started, there is no available drug for limiting its progression. However, there is one group in Canada working on a nutritional strategy, a ketogenic beverage. They reported that a ketogenic beverage increases brain energy metabolism in Alzheimer's patients [151, 152].

Returning to the question of if we are ready to change recommendations on n-3 FAs, we think that we are not there yet. However, working on the biology of aging might greatly improve our understanding about what are «normal» modifications occurring during aging and what can become pathological. Seizing this opportunity, we might contribute to the prevention of cognitive decline in the future with nutrition playing a vital role in this process.

Acknowledgements

This work was supported by Canadian Institute of Health Research (CIHR, MOP 119454), Quebec Network of research in aging, Natural Science and Engineering Research Council of Canada, Research center on aging for a scholarship to LC, Faculté de Médecine et des sciences de la santé for a scholarship to HCM, and CIHR for a salary award to MP. MP also hold an institutional research chair on lipid metabolism during aging funded by the medical research center of Université de Sherbrooke.

Conflicts of Interest

MP received funding from Neptune Wellness Solutions but the funding did not funded studies cited in this review and did not funded any of the work of this review.

References

1. Barberger-Gateau, P., et al., *Fish, meat, and risk of dementia: cohort study.* Bmj, 2002. **325**(7370): p. 932-3.
2. Holub, D.J. and B.J. Holub, *Omega-3 fatty acids from fish oils and cardiovascular disease.* Mol Cell Biochem, 2004. **263**(1-2): p. 217-25.
3. Morris, M.C., et al., *Fish consumption and cognitive decline with age in a large community study.* Arch Neurol, 2005. **62**(12): p. 1849-53.
4. Arterburn, L.M., E.B. Hall, and H. Oken, *Distribution, interconversion, and dose response of n-3 fatty acids in humans.* Am J Clin Nutr, 2006. **83**(6): p. S1467-1476.
5. Vidgren, H.M., et al., *Incorporation of n-3 fatty acids into plasma lipid fractions, and erythrocyte membranes and platelets during dietary supplementation with fish, fish oil, and docosahexaenoic acid-rich oil among healthy young men.* Lipids, 1997. **32**(7): p. 697-705.
6. Calder, P.C., *Polyunsaturated fatty acids and inflammation.* Prostaglandins Leukot Essent Fatty Acids, 2006. **75**(3): p. 197-202.
7. Plourde, M. and S.C. Cunnane, *Extremely limited synthesis of long chain polyunsaturates in adults: Implications for their dietary essentiality and use as suppements.* Appl Physiol Nutr Metab., 2007. **32**(4): p. 619-634.
8. Blasbalg, T.L., et al., *Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century.* The American Journal of Clinical Nutrition, 2011. **93**(5): p. 950-962.
9. Cunnane, S.C., et al., *Fish, docosahexaenoic acid and Alzheimer's disease.* Prog Lipid Res, 2009. **48**(5): p. 239-56.
10. Salem, N., Jr., M. Vandal, and F. Calon, *The benefit of docosahexaenoic acid for the adult brain in aging and dementia.* Prostaglandins Leukot Essent Fatty Acids, 2015. **92**: p. 15-22.
11. Barberger-Gateau, P., et al., *Dietary omega 3 polyunsaturated fatty acids and Alzheimer's disease: interaction with apolipoprotein E genotype.* Curr Alzheimer Res, 2011. **8**(5): p. 479-91.
12. Loef, M. and H. Walach, *The Omega-6/Omega-3 Ratio and Dementia or Cognitive Decline: A Systematic Review on Human Studies and Biological Evidence.* Journal of Nutrition in Gerontology and Geriatrics, 2013. **32**(1): p. 1-23.

13. van der Lee, S.J., et al., *Circulating metabolites and general cognitive ability and dementia: Evidence from 11 cohort studies.* Alzheimers Dement, 2018. **14**(6): p. 707-722.
14. Leveille, P., et al., *Metabolism of uniformly labeled 13C-eicosapentaenoic acid and 13C-arachidonic acid in young and old men.* Am J Clin Nutr, 2017.
15. Plourde, M., et al., *Plasma incorporation, apparent retroconversion and beta-oxidation of 13C-docosahexaenoic acid in the elderly.* Nutrition & metabolism, 2011. **8**: p. 5.
16. Mc Auley, M.T. and K.M. Mooney, *Computationally Modeling Lipid Metabolism and Aging: A Mini-review.* Comput Struct Biotechnol J, 2015. **13**: p. 38-46.
17. Schwenk, R.W., et al., *Fatty acid transport across the cell membrane: regulation by fatty acid transporters.* Prostaglandins, leukotrienes, and essential fatty acids, 2010. **82**(4-6): p. 149-54.
18. Mu, H. and C.E. Hoy, *The digestion of dietary triacylglycerols.* Prog Lipid Res, 2004. **43**(2): p. 105-33.
19. Mattson, F.H. and R.A. Volpenhein, *The Digestion and Absorption of Triglycerides.* J Biol Chem, 1964. **239**: p. 2772-7.
20. Kunisaki S and M. C., *Ultrasound growth patterns of fetal lung malformations: Implications on prenatal care and postnatal outcome.* Prenat Diagn., 2015. **35**(24): p. 89-90.
21. D'Aquila, T., et al., *Recent discoveries on absorption of dietary fat: Presence, synthesis, and metabolism of cytoplasmic lipid droplets within enterocytes.* Biochim Biophys Acta, 2016. **1861**(8 Pt A): p. 730-47.
22. Bisgaier, C.L. and R.M. Glickman, *Intestinal synthesis, secretion, and transport of lipoproteins.* Annu Rev Physiol, 1983. **45**: p. 625-36.
23. Mansbach, C.M., 2nd and P. Nevin, *Intracellular movement of triacylglycerols in the intestine.* J Lipid Res, 1998. **39**(5): p. 963-8.
24. Cooper, A.D., *Hepatic uptake of chylomicron remnants.* J Lipid Res, 1997. **38**(11): p. 2173-92.
25. Redgrave, T.G. and D.M. Small, *Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat.* J Clin Invest, 1979. **64**(1): p. 162-71.
26. Willnow, T.E., *Mechanisms of hepatic chylomicron remnant clearance.* Diabet Med, 1997. **14 Suppl 3**: p. S75-80.
27. Gruffat, D., et al., *Regulation of VLDL synthesis and secretion in the liver.* Reprod Nutr Dev, 1996. **36**(4): p. 375-89.
28. Havel, R.J., *The formation of LDL: mechanisms and regulation.* J Lipid Res, 1984. **25**(13): p. 1570-6.
29. Brown, M.S. and J.L. Goldstein, *A receptor-mediated pathway for cholesterol homeostasis.* Science, 1986. **232**(4746): p. 34-47.
30. Zhang, Y., et al., *Dysregulation of the Low-Density Lipoprotein Receptor Pathway Is Involved in Lipid Disorder-Mediated Organ Injury.* Int J Biol Sci, 2016. **12**(5): p. 569-79.
31. B., B., *Stress oxydant et pathologies cardiovasculaires. .* Médecine Thérapeutique Cardiol. , 2006. **2**(1): p. 43-52.
32. Fortier, M., et al., *Higher plasma n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related change in n-3 fatty acid metabolism?* Prostaglandins, leukotrienes, and essential fatty acids, 2010. **82**(4-6): p. 277-80.
33. Einarsson, K., et al., *Influence of age on secretion of cholesterol and synthesis of bile acids by the liver.* N Engl J Med, 1985. **313**(5): p. 277-82.
34. Lehto, S., et al., *Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM.* Diabetes, 1997. **46**(8): p. 1354-9.
35. Sears, B. and M. Perry, *The role of fatty acids in insulin resistance.* Lipids Health Dis, 2015. **14**: p. 121.
36. Toth, M.J. and A. Tchernof, *Lipid metabolism in the elderly.* Eur J Clin Nutr, 2000. **54 Suppl 3**: p. S121-5.
37. DeFronzo, R.A., *Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM.* Diabetes, 1988. **37**(6): p. 667-87.
38. Fink, R.I., et al., *Mechanisms of insulin resistance in aging.* J Clin Invest, 1983. **71**(6): p. 1523-35.
39. Verges, B., *Pathophysiology of diabetic dyslipidaemia: where are we?* Diabetologia, 2015. **58**(5): p. 886-99.

40. Austin, M.A., J.E. Hokanson, and K.L. Edwards, *Hypertriglyceridemia as a cardiovascular risk factor.* Am J Cardiol, 1998. **81**(4A): p. 7B-12B.
41. Ericsson, S., et al., *The influence of age on low density lipoprotein metabolism: effects of cholestyramine treatment in young and old healthy male subjects.* J Intern Med, 1997. **242**(4): p. 329-37.
42. Russell, D.W. and K.D. Setchell, *Bile acid biosynthesis.* Biochemistry, 1992. **31**(20): p. 4737-49.
43. Grundy, S.M., et al., *Cholesterol lowering in the elderly population. Coordinating Committee of the National Cholesterol Education Program.* Arch Intern Med, 1999. **159**(15): p. 1670-8.
44. Fernandez, M.L. and K.L. West, *Mechanisms by which dietary fatty acids modulate plasma lipids.* J Nutr, 2005. **135**(9): p. 2075-8.
45. Christie, W.W., *Rapid separation and quantification of lipid classes by high performance liquid chromatography and mass (light-scattering) detection.* J Lipid Res, 1985. **26**(4): p. 507-12.
46. Hodson, L., C.M. Skeaff, and B.A. Fielding, *Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake.* Prog Lipid Res, 2008. **47**(5): p. 348-80.
47. Block, R.C., W.S. Harris, and J.V. Pottala, *Determinants of Blood Cell Omega-3 Fatty Acid Content.* Open Biomark J, 2008. **1**: p. 1-6.
48. Gellert, S., J.P. Schuchardt, and A. Hahn, *Low long chain omega-3 fatty acid status in middle-aged women.* Prostaglandins Leukot Essent Fatty Acids, 2017. **117**: p. 54-59.
49. Harris, W.S., et al., *Red blood cell polyunsaturated fatty acids and mortality in the Women's Health Initiative Memory Study.* J Clin Lipidol, 2017. **11**(1): p. 250-259 e5.
50. Harris, W.S., et al., *Clinical correlates and heritability of erythrocyte eicosapentaenoic and docosahexaenoic acid content in the Framingham Heart Study.* Atherosclerosis, 2012. **225**(2): p. 425-31.
51. Harris, W.S., et al., *Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: observations from 160,000 patients.* Prostaglandins Leukot Essent Fatty Acids, 2013. **88**(4): p. 257-63.
52. Sands, S.A., et al., *The impact of age, body mass index, and fish intake on the EPA and DHA content of human erythrocytes.* Lipids, 2005. **40**(4): p. 343-7.
53. Aarsetoey, H., et al., *(n-3) Fatty acid content of red blood cells does not predict risk of future cardiovascular events following an acute coronary syndrome.* J Nutr, 2009. **139**(3): p. 507-13.
54. Block, R.C., et al., *EPA and DHA in blood cell membranes from acute coronary syndrome patients and controls.* Atherosclerosis, 2008. **197**(2): p. 821-8.
55. Caprari, P., et al., *Aging and red blood cell membrane: a study of centenarians.* Exp Gerontol, 1999. **34**(1): p. 47-57.
56. Carver, J.D., et al., *The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects.* Brain Res Bull, 2001. **56**(2): p. 79-85.
57. Farzaneh-Far, R., et al., *Inverse association of erythrocyte n-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study.* Atherosclerosis, 2009. **205**(2): p. 538-43.
58. Itomura, M., et al., *Factors influencing EPA+DHA levels in red blood cells in Japan.* In Vivo, 2008. **22**(1): p. 131-5.
59. Thorlaksdottir, A.Y., et al., *Positive association between plasma antioxidant capacity and n-3 PUFA in red blood cells from women.* Lipids, 2006. **41**(2): p. 119-25.
60. Yanagisawa, N., et al., *Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area.* J Atheroscler Thromb, 2010. **17**(3): p. 285-94.
61. Brenna, J.T., et al., *Best practices for the design, laboratory analysis, and reporting of trials involving fatty acids.* Am J Clin Nutr, 2018. **108**(2): p. 211-227.
62. Asciutti-Moura, L.S., et al., *Fatty acid composition of serum lipids and its relation to diet in an elderly institutionalized population.* Am J Clin Nutr, 1988. **48**(4): p. 980-7.
63. Crowe, F.L., et al., *Serum n-3 long-chain PUFA differ by sex and age in a population-based survey of New Zealand adolescents and adults.* Br J Nutr, 2008. **99**(1): p. 168-74.

64. de Groot, R.H., et al., *Age dependence of plasma phospholipid fatty acid levels: potential role of linoleic acid in the age-associated increase in docosahexaenoic acid and eicosapentaenoic acid concentrations.* Br J Nutr, 2009. **102**(7): p. 1058-64.
65. Dewailly, E., et al., *Cardiovascular disease risk factors and n-3 fatty acid status in the adult population of James Bay Cree.* Am J Clin Nutr, 2002. **76**(1): p. 85-92.
66. Dewailly, E., et al., *n-3 Fatty acids and cardiovascular disease risk factors among the Inuit of Nunavik.* Am J Clin Nutr, 2001. **74**(4): p. 464-73.
67. Dewailly, E.E., et al., *Relations between n-3 fatty acid status and cardiovascular disease risk factors among Quebecers.* Am J Clin Nutr, 2001. **74**(5): p. 603-11.
68. Ogura, T., et al., *Fatty acid composition of plasma, erythrocytes and adipose: their correlations and effects of age and sex.* Lipids, 2010. **45**(2): p. 137-44.
69. Nogi, A., et al., *Plasma n-3 Polyunsaturated Fatty Acid and Cardiovascular Disease Risk Factors in Japanese, Korean and Mongolian Workers.* Journal of Occupational Health, 2007. **49**(3): p. 205-216.
70. Skuladottir, G.V., et al., *Plasma fatty acids and lipids in two separate, but genetically comparable, Icelandic populations.* Lipids, 1995. **30**(7): p. 649-55.
71. Stark, K.D., E.J. Park, and B.J. Holub, *Fatty acid composition of serum phospholipid of premenopausal women and postmenopausal women receiving and not receiving hormone replacement therapy.* Menopause, 2003. **10**(5): p. 448-55.
72. Fortier, M., et al., *Higher plasma n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related change in n-3 fatty acid metabolism?* Prostaglandins Leukot Essent Fatty Acids, 2010. **82**(4-6): p. 277-80.
73. Meydani, M., et al., *Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women.* J Nutr, 1991. **121**(4): p. 484-91.
74. Meydani, S.N., et al., *Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women.* J Nutr, 1991. **121**(4): p. 547-55.
75. Plourde, M., et al., *Eicosapentaenoic acid decreases postprandial beta-hydroxybutyrate and free fatty acid responses in healthy young and elderly.* Nutrition, 2009. **25**(3): p. 289-94.
76. Rees, D., et al., *Dose-related effects of eicosapentaenoic acid on innate immune function in healthy humans: a comparison of young and older men.* Am J Clin Nutr, 2006. **83**(2): p. 331-42.
77. Vandal, M., et al., *Plasma omega-3 fatty acid response to a fish oil supplement in the healthy elderly.* Lipids, 2008. **43**(11): p. 1085-9.
78. Walker, C.G., et al., *Age and sex differences in the incorporation of EPA and DHA into plasma fractions, cells and adipose tissue in humans.* Br J Nutr, 2014. **111**(4): p. 679-89.
79. Flock, M.R., et al., *Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial.* J Am Heart Assoc, 2013. **2**(6): p. e000513.
80. Witt, P.M., et al., *The incorporation of marine n-3 PUFA into platelets and adipose tissue in pre- and postmenopausal women: a randomised, double-blind, placebo-controlled trial.* Br J Nutr, 2010. **104**(3): p. 318-25.
81. Kiortsis, D.N., et al., *Factors associated with low compliance with lipid-lowering drugs in hyperlipidemic patients.* Journal of Clinical Pharmacy and Therapeutics, 2000. **25**(6): p. 445-451.
82. Brossard, N., et al., *Human plasma albumin transports [13C]docosahexaenoic acid in two lipid forms to blood cells.* J Lipid Res, 1997. **38**(8): p. 1571-82.
83. Brossard, N., et al., *Retroconversion and metabolism of [13C]22:6n-3 in humans and rats after intake of a single dose of [13C]22:6n-3-triacylglycerols.* Am J Clin Nutr, 1996. **64**(4): p. 577-86.
84. Lemaitre-Delaunay, D., et al., *Blood compartmental metabolism of docosahexaenoic acid (DHA) in humans after ingestion of a single dose of [(^{13})C]DHA in phosphatidylcholine.* J Lipid Res, 1999. **40**(10): p. 1867-74.
85. Fortier, M., et al., *Higher plasma n-3 fatty acid status in the moderately healthy elderly in southern Quebec: higher fish intake or aging-related change in n-3 fatty acid metabolism?* Prostaglandins Leukot Essent Fatty Acids. **82**(4-6): p. 277-80.

86. Vandal, M., et al., *Plasma omega-3 fatty acid response to a fish oil supplement in the healthy elderly.* Lipids, 2008. **43**(11): p. 1085-1089.
87. Hedman, A.M., et al., *Human brain changes across the life span: a review of 56 longitudinal magnetic resonance imaging studies.* Hum Brain Mapp, 2012. **33**(8): p. 1987-2002.
88. Coffey, C.E., et al., *Sex differences in brain aging: a quantitative magnetic resonance imaging study.* Arch Neurol, 1998. **55**(2): p. 169-79.
89. Murphy, D.G., et al., *Sex differences in human brain morphometry and metabolism: an in vivo quantitative magnetic resonance imaging and positron emission tomography study on the effect of aging.* Arch Gen Psychiatry, 1996. **53**(7): p. 585-94.
90. Bourisly, A.K., et al., *A voxel-based morphometric magnetic resonance imaging study of the brain detects age-related gray matter volume changes in healthy subjects of 21-45 years old.* Neuroradiol J, 2015. **28**(5): p. 450-9.
91. Draganski, B., et al., *Regional specificity of MRI contrast parameter changes in normal ageing revealed by voxel-based quantification (VBQ).* Neuroimage, 2011. **55**(4): p. 1423-34.
92. Hafkemeijer, A., et al., *Associations between age and gray matter volume in anatomical brain networks in middle-aged to older adults.* Aging Cell, 2014. **13**(6): p. 1068-74.
93. Jancke, L., et al., *Brain size, sex, and the aging brain.* Hum Brain Mapp, 2015. **36**(1): p. 150-69.
94. Minkova, L., et al., *Gray matter asymmetries in aging and neurodegeneration: A review and meta-analysis.* Hum Brain Mapp, 2017. **38**(12): p. 5890-5904.
95. Peng, F., et al., *A Cross-Sectional Voxel-Based Morphometric Study of Age- and Sex-Related Changes in Gray Matter Volume in the Normal Aging Brain.* J Comput Assist Tomogr, 2016. **40**(2): p. 307-15.
96. Tremblay, P., A.S. Dick, and S.L. Small, *Functional and structural aging of the speech sensorimotor neural system: functional magnetic resonance imaging evidence.* Neurobiol Aging, 2013. **34**(8): p. 1935-51.
97. Callaghan, M.F., et al., *Widespread age-related differences in the human brain microstructure revealed by quantitative magnetic resonance imaging.* Neurobiol Aging, 2014. **35**(8): p. 1862-72.
98. Honda, Y., et al., *Volumetric analyses of cerebral white matter hyperintensity lesions on magnetic resonance imaging in a Japanese population undergoing medical check-up.* Geriatr Gerontol Int, 2015. **15 Suppl 1**: p. 43-7.
99. Liu, H., et al., *A voxel-based morphometric study of age- and sex-related changes in white matter volume in the normal aging brain.* Neuropsychiatr Dis Treat, 2016. **12**: p. 453-65.
100. Coffler, M.S., et al., *Enhanced granulosa cell responsiveness to follicle-stimulating hormone during insulin infusion in women with polycystic ovary syndrome treated with pioglitazone.* The Journal of clinical endocrinology and metabolism, 2003. **88**(12): p. 5624-31.
101. Xu, J., et al., *Gender effects on age-related changes in brain structure.* AJNR Am J Neuroradiol, 2000. **21**(1): p. 112-8.
102. Van Der Werf, Y.D., et al., *Thalamic volume predicts performance on tests of cognitive speed and decreases in healthy aging. A magnetic resonance imaging-based volumetric analysis.* Brain Res Cogn Brain Res, 2001. **11**(3): p. 377-85.
103. Erten-Lyons, D., et al., *Neuropathologic basis of age-associated brain atrophy.* JAMA Neurol, 2013. **70**(5): p. 616-22.
104. Fjell, A.M., et al., *Critical ages in the life course of the adult brain: nonlinear subcortical aging.* Neurobiol Aging, 2013. **34**(10): p. 2239-47.
105. Fraser, M.A., M.E. Shaw, and N. Cherbuin, *A systematic review and meta-analysis of longitudinal hippocampal atrophy in healthy human ageing.* Neuroimage, 2015. **112**: p. 364-374.
106. Onozuka, M., et al., *Age-related changes in brain regional activity during chewing: a functional magnetic resonance imaging study.* J Dent Res, 2003. **82**(8): p. 657-60.
107. Heuninckx, S., N. Wenderoth, and S.P. Swinnen, *Systems neuroplasticity in the aging brain: recruiting additional neural resources for successful motor performance in elderly persons.* J Neurosci, 2008. **28**(1): p. 91-9.

108. Holtzer, R., et al., *Neuroimaging of mobility in aging: a targeted review.* J Gerontol A Biol Sci Med Sci, 2014. **69**(11): p. 1375-88.
109. Kim, J.H., et al., *Functional magnetic resonance imaging reveals age-related alterations to motor networks in weighted elbow flexion-extension movement.* Neurol Res, 2010. **32**(9): p. 995-1001.
110. Linortner, P., et al., *Aging associated changes in the motor control of ankle movements in the brain.* Neurobiol Aging, 2014. **35**(10): p. 2222-2229.
111. Gunning-Dixon, F.M. and N. Raz, *The cognitive correlates of white matter abnormalities in normal aging: a quantitative review.* Neuropsychology, 2000. **14**(2): p. 224-32.
112. Yuan, P. and N. Raz, *Prefrontal cortex and executive functions in healthy adults: a meta-analysis of structural neuroimaging studies.* Neurosci Biobehav Rev, 2014. **42**: p. 180-92.
113. Van Petten, C., *Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis.* Neuropsychologia, 2004. **42**(10): p. 1394-413.
114. O'Shea, A., et al., *Cognitive Aging and the Hippocampus in Older Adults.* Front Aging Neurosci, 2016. **8**: p. 298.
115. Gonneaud, J., et al., *Functional dedifferentiation and reduced task-related deactivations underlie the age-related decline of prospective memory.* Brain Imaging Behav, 2017. **11**(6): p. 1873-1884.
116. Meusel, L.A., et al., *Brain-behavior relationships in source memory: Effects of age and memory ability.* Cortex, 2017. **91**: p. 221-233.
117. Rieckmann, A., S. Pudas, and L. Nyberg, *Longitudinal Changes in Component Processes of Working Memory.* eNeuro, 2017. **4**(2).
118. Suzuki, Y., et al., *Functional magnetic resonance imaging of odor identification: the effect of aging.* J Gerontol A Biol Sci Med Sci, 2001. **56**(12): p. M756-60.
119. Fusar-Poli, P., et al., *Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies.* J Psychiatry Neurosci, 2009. **34**(6): p. 418-32.
120. Grady, C.L., *Age-related differences in face processing: a meta-analysis of three functional neuroimaging experiments.* Can J Exp Psychol, 2002. **56**(3): p. 208-20.
121. Daneault, V., et al., *Aging reduces the stimulating effect of blue light on cognitive brain functions.* Sleep, 2014. **37**(1): p. 85-96.
122. Sala-Llonch, R., et al., *Changes in whole-brain functional networks and memory performance in aging.* Neurobiol Aging, 2014. **35**(10): p. 2193-202.
123. Tomasi, D. and N.D. Volkow, *Aging and functional brain networks.* Mol Psychiatry, 2012. **17**(5): p. 471, 549-58.
124. Archer, J.A., et al., *A Comprehensive Analysis of Connectivity and Aging Over the Adult Life Span.* Brain Connect, 2016. **6**(2): p. 169-85.
125. Geerligs, L., et al., *Reduced specificity of functional connectivity in the aging brain during task performance.* Hum Brain Mapp, 2014. **35**(1): p. 319-30.
126. Zhang, H., A. Lee, and A. Qiu, *A posterior-to-anterior shift of brain functional dynamics in aging.* Brain Struct Funct, 2017. **222**(8): p. 3665-3676.
127. Naber, D. and H.G. Dahnke, *Protein and nucleic acid content in the aging human brain.* Neuropathol Appl Neurobiol, 1979. **5**(1): p. 17-24.
128. Soderberg, M., et al., *Lipid compositions of different regions of the human brain during aging.* Journal of neurochemistry, 1990. **54**(2): p. 415-23.
129. Surowka, A.D., et al., *Variability of protein and lipid composition of human subtantia nigra in aging: Fourier transform infrared microspectroscopy study.* Neurochem Int, 2014. **76**: p. 12-22.
130. Svennerholm, L., et al., *Membrane lipids of adult human brain: lipid composition of frontal and temporal lobe in subjects of age 20 to 100 years.* Journal of neurochemistry, 1994. **63**(5): p. 1802-11.
131. Hancock, S.E., et al., *The phospholipid composition of the human entorhinal cortex remains relatively stable over 80 years of adult aging.* Geroscience, 2017. **39**(1): p. 73-82.

132. Norris, S.E., et al., *Human prefrontal cortex phospholipids containing docosahexaenoic acid increase during normal adult aging, whereas those containing arachidonic acid decrease.* Neurobiol Aging, 2015. **36**(4): p. 1659-1669.
133. Yassine, H.N., et al., *DHA brain uptake and APOE4 status: a PET study with [1-(11)C]-DHA.* Alzheimers Res Ther, 2017. **9**(1): p. 23.
134. Coon, K.D., et al., *A high-density whole-genome association study reveals that APOE is the major susceptibility gene for sporadic late-onset Alzheimer's disease.* J Clin Psychiatry, 2007. **68**(4): p. 613-8.
135. Chouinard-Watkins, R., et al., *Interactive impact of BMI and APOE genotype on the plasma long chain polyunsaturated fatty acid response to a fish oil supplement in healthy participants* Am J Clin Nutr, 2015. **102**: p. 505-513.
136. Chouinard-Watkins, R., et al., *Disturbance in uniformly 13C-labelled DHA metabolism in elderly human subjects carrying the apoE epsilon4 allele.* Br J Nutr, 2013. **110**(10): p. 1751-9.
137. Conway, V., et al., *Postprandial enrichment of triacylglycerol-rich lipoproteins with omega-3 fatty acids: lack of an interaction with apolipoprotein E genotype?* Lipids in health and disease, 2014. **13**(1): p. 148.
138. Plourde, M., et al., *Plasma n-3 fatty acid response to an n-3 fatty acid supplement is modulated by apoE epsilon4 but not by the common PPAR-alpha L162V polymorphism in men.* Br J Nutr, 2009. **102**(8): p. 1121-4.
139. Nock, T.G., R. Chouinard-Watkins, and M. Plourde, *Carriers of an apolipoprotein E epsilon 4 allele are more vulnerable to a dietary deficiency in omega-3 fatty acids and cognitive decline.* Biochim Biophys Acta, 2017. **1862**(10 Pt A): p. 1068-1078.
140. Gu, Y., et al., *White matter integrity as a mediator in the relationship between dietary nutrients and cognition in the elderly.* Ann Neurol, 2016. **79**(6): p. 1014-25.
141. Raji, C.A., et al., *Regular fish consumption and age-related brain gray matter loss.* Am J Prev Med, 2014. **47**(4): p. 444-51.
142. Samieri, C., et al., *Plasma long-chain omega-3 fatty acids and atrophy of the medial temporal lobe.* Neurology, 2012. **79**(7): p. 642-50.
143. Witte, A.V., et al., *Long-chain omega-3 fatty acids improve brain function and structure in older adults.* Cereb Cortex, 2014. **24**(11): p. 3059-68.
144. Salthouse, T.A., D.R. Mitchell, and R. Palmon, *Memory and age differences in spatial manipulation ability.* Psychol Aging, 1989. **4**(4): p. 480-6.
145. Salthouse, T.A., et al., *Effects of adult age and working memory on reasoning and spatial abilities.* J Exp Psychol Learn Mem Cogn, 1989. **15**(3): p. 507-16.
146. Blennow, K., M.J. de Leon, and H. Zetterberg, *Alzheimer's disease.* Lancet, 2006. **368**(9533): p. 387-403.
147. Deary, I.J., et al., *Cognitive change and the APOE epsilon 4 allele.* Nature, 2002. **418**(6901): p. 932.
148. Hoyer, S., *The aging brain. Changes in the neuronal insulin/insulin receptor signal transduction cascade trigger late-onset sporadic Alzheimer disease (SAD). A mini-review.* J Neural Transm, 2002. **109**(7-8): p. 991-1002.
149. Rosenberg, A., et al., *Multidomain lifestyle intervention benefits a large elderly population at risk for cognitive decline and dementia regardless of baseline characteristics: The FINGER trial.* Alzheimers Dement, 2018. **14**(3): p. 263-270.
150. Lehtisalo, J., et al., *Dietary changes and cognition over 2 years within a multidomain intervention trial-The Finnish Geriatric Intervention Study to Prevent Cognitive Impairment and Disability (FINGER).* Alzheimers Dement, 2018.
151. Croteau, E., et al., *A cross-sectional comparison of brain glucose and ketone metabolism in cognitively healthy older adults, mild cognitive impairment and early Alzheimer's disease.* Exp Gerontol, 2018. **107**: p. 18-26.
152. Croteau, E., et al., *Ketogenic Medium Chain Triglycerides Increase Brain Energy Metabolism in Alzheimer's Disease.* J Alzheimers Dis, 2018. **64**(2): p. 551-561.

Table 1: Cross-sectional modulation by age of blood fatty acid

				Age-increasing effects at baseline in blood pool		
Reference	***n*, sex and age**	**Blood pool**	**Age-increasing effects**	**Omega-3 index**	**EPA**	**DHA**
[53]	n=460, 299 males and 161 females, 29-97 y (~72 y)	RBC	9.8 y older in Higher Omega-3 Index Quartile compared to Lower Quartile	Higher Omega-3 Index quartile was 9.8 y older vs lower quartile		
[62]	53 institutionalized elderly subjects (24 males and 29 females), ≥ 60 y (~ 79 y); 25 young healthy adults (8 males and 17 females), 20 – 42 y (~29 y)	Serum NEFA, TG, CE and PL	In serum PL: EPA higher in elderly; DHA and DPA: appear lower in the elderly but not significantly different		PL: 114% higher in elderly group	
[54]	768 acute coronary syndrome patients and 768 matched controls (66 % male, ~61 y)	RBC membranes	Positive relation between age and EPA and DHA levels: 8 years older in those with higher EPA + DHA levels vs those with lower group		Higher RBC EPA + DHA group: 8 y older compared to Lower group	Higher RBC EPA + DHA group: 8 y older compared to Lower group
[47]	704 outpatients (67% male), ~62 y	RBC	RBC Omega-3 Index increases with age	5.3% increase per 10-year age increase		
[55]	15 centenarians (12 females and 3 males),~ 103 y (101–107 y), living in a family unit, self-sufficient and without major illnesses and 13 normal subjects (6 males and 7 females), ~65 y (60 – 69 y)	RBC PL	Increased DHA in RBC-PC and in RBC-PE, and increased DPA in RBC-PS and RBC-PE;			PC: 116% higher in centenarian group PE: 60% higher in centenarian group

[63]	2793 New Zealanders ≥15 y (45% males, 55% females)	Serum PL, CE and TG	Serum PL: EPA and DHA increase with age in both sexes while DPA increases with age only in women aged between of 20 and 73 y		PL: in both sexes, increased by 0.31 mol% between 20 and 73 y	PL: in both sexes, increased by 0.28 mol% between 20 and 73 y
[64]	234 males and females (Dutch: low fish consumption), 36 to 88 years (~60 y)	Plasma PL	Significant positive relationship between age and plasma PL concentrations of DHA and EPA		~~PL: ~1.5 fold increased between 36 to 88 y~~	~~PL: ~1.3 fold increased between 36 to 88 y~~
[66]	426 Inuits, 18 to 74 years: 179 males (~38.7 y) and 247 females (~37.8 y), n=254 in 18-39 y and n=172 in ≥40 y	Plasma PL	EPA: AA and n-3: n-6 ratios, and relative concentrations of EPA, DHA, and EPA + DHA increased significantly with age		139% higher in ≥40 y group compared to 18-39 y group	43% higher in ≥40 y group compared to 18-39 y group
[67]	1460 subjects, 18–74 years: 722 males (~40.6 y) and 738 females (~39.6 y), n=784 in 18-34 y, n=432 in 35-49 y and n=244 in 50-74 y	Plasma PL	Older persons had higher EPA, DHA, EPA+DHA, EPA: AA and n-3: n-6 ratio in older vs younger individuals		14% higher in 50-74 y group compared to 18-34 y	19% higher in 50-74 y group compared to 18-34 y
[65]	917 subjects, 18-74 y: 422 males (~36.0 y) and 495 females (~35.6 y), n=536 in 18-34 y, n=220 in 35-49 y and n=161 in 50-74 y	Plasma PL	EPA: AA, n-3: n-6 FA, and relative concentrations of EPA, DHA, and EPA+DHA did not vary according to sex, but they significantly increased ~~in the concentrations~~ with age		146% higher in 50-74 y group compared to 18-34 y	67% higher in 50-74 y group compared to 18-34 y
[57]	992 participants (mainly males: >80%), age: early 50s to late 70s	RBC membranes	Lower levels EPA + DHA were significantly associated with younger age			

[48]	446 females, ~48,5 y (40–60 y)	RBC membrane	In females aged ≥50 years, EPA and DPA levels and omega-3 index were significantly higher compared to females under the age of 50 years.	4% higher in ≥50 y compared to <50 y	13% higher in ≥50 y compared to <50 y	
[50]	n= 3196, 55 % females, ~66 y (40-74 y)	RBC	RBC Omega-3 Index increases with age	5% increase every decade		
[51]	159 771 patients (48% males, 52% females) being evaluated by their physicians for CVD risk	RBC	Increases in EPA and DHA each decade. After age 70, significant decrease in EPA while DHA remains stable	7% increase by decade until 70 y, stable thereafter	13% increase by decade up to 70 y, then 9% decrease each decade thereafter	6% increase by decade until 70 y, stable thereafter
[49]	6501 females aged 65–80, ~15 years follow-up	RBC membrane	RBC Omega-3 Index increases with age	Higher Omega-3 index quartile: 0.6 y older compared to lower quartile		
[58]	n=456, 320 males and 136 females, 18 to 70 y (~42.5 y)	RBC PL	~~EPA+DHA: ~1.4 fold increase in both gender between 18-20 vs 60+ years~~ Age was significantly correlated with RBC PL EPA+DHA levels			
[69]	411 Japanese (194 males and 217 females), 418 Koreans (240 males and 178 females) and 252 Mongolians (100 males and 152 females) aged 30-60 y	Plasma	EPA and DHA increase with age in the Japanese and Koreans. For Mongolians, there was a significant decrease in DHA and no significant change in EPA with age (50-60 y vs. 30-39 y)		Japanese: 44% increase Koreans: 86% increase	Japanese: 16% increase Koreans: 28% increase Mongolians: 9% decrease
[68]	75 adults admitted for elective surgery: 48 males (~58 y: 27-81 y) and 27 females (~58 y:	Plasma PL, RBC PL and AT TG	Positive correlation between EPA+DHA and age in plasma and RBC PL but not in AT TG			

	33-74 y)					
[52]	163 adults, 74 males and 89 females, 20 to 80 years	RBC	Omega-3 Index increased each ~~decade but decreased by 0.3 units with each 3-unit increase in BMI~~	0.5 unit increase by 10 years of age		
[70]	119 subjects for each population, Icelandic (59 males and 60 females) and Icelandic-Canadians (60 males and 59 females), 20-69 years	Plasma PL	Young Icelandic-Canadians had lower levels of EPA than the middle and oldest age groups		80% increase in oldest group (60-69.9 y) compared to the youngest (20-39.9 y)	
[71]	54 females, 43-60 years: 19 premenopausal (~48 y), 16 postmenopausal not receiving HRT (~52 y) and 19 postmenopausal receiving HRT (~51 y)	Serum PL	DHA levels were significantly lower in premenopausal females than postmenopausal females not receiving HRT. DHA levels did not differ significantly between premenopausal females and postmenopausal females receiving HRT. Those receiving HRT had significantly lower levels of DPA.			34% increase in postmenopausal women without HRT vs premenopausal
	~~338 women; alcohol intake: abstainers (n=254, ~24,2 y), occasional (n=45, ~27,9 y) and habitual (n=8, ~30,5 y)~~	~~Plasma and RBC~~	~~DHA and AA correlates positively with maternal age~~			~~↑ in plasma (µg/ml et %) ↑ in RBC (%)~~
[59]	99 Icelandic females, 18 to 73 y (~45.8 y)	RBC	Proportions of total n-3 PUFA, EPA, and DHA correlated positively with age		↑	↑

[60]	200 Japanese, 126 males and 74 females, ~50 y (<35 to ≥65 y)	Serum and RBC total lipids	EPA, DHA, n-3: n-6 ratio and EPA: AA ratio increased with age ~~(stronger effect in serum):~~		Group ≥65 y compared to group <35 y: 127% increase in EPA concentration in serum and 100% increase in relative percentage of EPA in RBC Group ≥65 y compared to group <35 y: 60% increase in DHA concentration in serum and 17% increase in relative percentage of DHA in RBC

AA: arachidonic acid, EPA: eicosapentaenoic acid: DHA, docosahexaenoic acid: DPA: docosapentaenoic acid, AT: adipose tissue, PUFA: polyunsaturated fatty acids, FA: fatty acids, PC: phosphatidylcholine, PE: phosphatidylethanolamine, PS: phosphatidylserine, TG: triglycerides, CE: cholesteryl esters, NEFA: non-esterified fatty acids, RBC: red blood cells, HRT: Hormone receiving therapy, BMI: body mass index

Table 2: Blood fatty acid modulation by age after an omega-3 fatty acid supplementation

Reference	***n*, sex and age**	**Blood pool**	**Omega-3 supplementation**	**Age effects**
[79]	n=115, 60 males and 55 females, 20 to 45 years	RBC	1 of 5 doses (0, 300, 600, 900, 1800 mg/day) of EPA+DHA (fish oil) for 5 months	Lower Omega-3 Index (O3I) status (P<0.0001) and older age (P=0.02) each predicted greater increases in O3I with supplementation
[72]	24–28 participants in each age group (except as noted in the tables), young adult = 18-30 y (~23 y) and elderly group = ≥65 y (~74 y)	Plasma	Two supplementations: n-3 supplement enriched in DHA (680 mg DHA/d plus 323 mg EPA/d) for 3 weeks, or a supplement enriched in EPA (1480 mg EPA/d plus 250 mg DHA/d) for 6 weeks	Expressed as % of total fatty acids: At baseline, total n-3 PUFA, EPA and DPA higher in elderly (32%, 100% and 25% respectively); Expressed as concentration (mg/L): At baseline, total n-3 PUFA, 18:3n-3, DHA, DPA and EPA higher in elderly (74%, 40%, 63%, 85% and 142% respectively); After supplementation: no higher effect with increasing age
[73]	14 young (22-35 y) and 9 older (51-71 y) females	Plasma	Daily 1680 mg EPA and 720 mg DHA for 3 months	Older women had a significantly higher increase in EPA and DHA than did young women (EPA: 932% increase in older group vs 700% increase in younger group and DHA: 156% increase in older group vs 98% increase in younger group)
[74]	6 young (23-33 y) and 6 older (51-68 y) females	Plasma	Daily 1680 mg EPA and 720 mg DHA for 3 months	At baseline there was no difference in percentage of EPA and DHA between young and older females; however, after 3 months of n-3 fatty acid supplementation, older females had a significantly higher increase in percentage of EPA and DHA compared to young females (EPA: 900% increase in older group vs 463% increase in younger group; DHA: 144% increase in older group vs 64% increase in younger group)
[75]	10 young (5 males and 5 females, ~22 y) and 10 elderly (5 males and 5 females, ~75 y)	Plasma	EPA-enriched supplement (1480 mg/d of EPA and 250 mg/d of DHA) for 6 weeks	Before and after the EPA supplement, fasting plasma EPA was higher in the elderly (by 85% and 67%, respectively)

[76]	Young (18-42 y; n=93) and old (53-70 y; n=62) males	Plasma and MNC PL	Placebo (corn oil) or 1.35 g EPA + 0.3 g DHA, 2.7 g EPA + 0.6 g DHA, or 4.05 g EPA + 0.9 g DHA per day for 12 weeks	In both plasma and MNC PL: at baseline, EPA and DHA increase with age, while after supplementation only EPA increases in old males. At baseline, EPA, DPA and DHA were ~33%, ~10% and ~43% higher, respectively, in plasma of older group; Baseline EPA and DHA were ~32% and ~26% higher, respectively, in MNC of older group. After high-EPA supplementation, EPA and DPA were ~49% and ~32% higher in plasma of older group and EPA was ~41% higher in MNC of older group
[77]	Elderly (n=10, 5 males and 5 females, ~74 y) and young (n=9, 5 males and 5 females, ~24 y)	Plasma total lipids, PL, TG, NEFA	680 mg/day of DHA and 323 mg/day of EPA for 3 weeks, followed by a 2-week wash-out during which no capsules were consumed	Higher baseline plasma EPA in elderly group; In response to the supplement, plasma DHA rose 42% more in the elderly vs the young group; EPA rose similarly in both groups
[78]	n=193 (101 females, 92 males), 20–79 y (young	Plasma PC, CE, NEFA and TG; MNC; RBC membranes; PLAT; BU; AT	EPA+DHA equivalent to 0, 1, 2 or 4 portions of oily fish per week, for 12 months (One portion consists of 1.5 g EPA + 1.77 g DHA)	At baseline, EPA in AT and DHA in plasma TG and AT were higher with increasing age; Following supplementation, EPA was higher in plasma T~~AG higher~~ with increasing age and DHA was lower in AT ~~smaller~~ with increasing age
[80]	89 Danish women: 45 premenopausal (~43 y) and 44 postmenopausal (~56 y), 18-70 y	PLAT, AT	2,2 g of marine n-3 PUFA (38,5% EPA, 25,9% DHA and 6,0% DPA) or control oil (thistle oil) daily for 12 weeks	Baseline contents of EPA, DPA and DHA were all significantly higher ~~(P<0.05)~~ in the PLAT (P<0.05) and AT (P<0.001) of the postmenopausal group, except for the content of EPA in PLAT (P=0.05); After supplementation, EPA, DPA, and DHA increase in PLAT and AT was~~, however,~~ the same in both groups

EPA: eicosapenaenoic acid, DHA, docosahexaenoic acid, DPA: docosapentaenoic acid, PLAT: platelets, AT: adipose tissue, PUFA: polyunsaturated fatty acids, PC: phosphatidylcholine, CE: cholesteryl esters, NEFA: non-esterified fatty acids, MNC: mononuclear cells, RBC: red blood cells, BU: buccal cells,