

1 *Review*

2 **Omega-3 PUFA metabolism and brain modifications during aging**

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14 **Abstract:**

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

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

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33 1. Introduction

34 Almost every country in the world experiences an aging population, and this population is expected to be
35 one of the most significant forces shaping our economy and society in the next 20-30 years. A major concern
36 about old age, both at the individual and societal levels, is a decline in health, especially if this means a loss
37 of self-sufficiency and independence. Increasing research aimed at promoting healthy aging is actually
38 ongoing but one of the major hurdles is to define the biology of aging. Aging in humans refers to a
39 multidimensional process of physical, psychological, and social changes. Therefore, it follows that
40 fundamental knowledge on the biological processes occurring during aging may help to design
41 environmental strategies aimed at promoting healthy biological aging. Thus, there is a need for better
42 prevention strategies, but one major gap in this field is a need to better understand what the biological
43 modifications are, also called geroscience, since this field is relatively new. One of the strategies to promote
44 healthy aging is the consumption of one or two fish meals each week¹⁻³. Normally, the intake of fish
45 positively correlates with increased plasma and erythrocyte omega-3 fatty acids (n-3 FA), likely with
46 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in a time- and dose-
47 dependent manner⁴⁻⁶. EPA and DHA have to be provided through the diet because their synthesis from their
48 precursor alpha-linolenic acid (ALA) is extremely limited in humans⁷. However, over the 20th century, the
49 dietary fat consumption has drastically changed with an increased level of omega-6 fatty acids such as
50 linoleic acid (LA) from 2.79% to 7.21% of energy. This shift in our dietary fat intake was largely due to our
51 dependence on new food production methodologies, including soybean oil⁸.

52 The link between our dietary fat intake and the incidence of chronic diseases has been largely debated over
53 the last 20 years. Our research group is mainly focused on prevention of cognitive decline, so the focus of
54 this review paper, with respect to chronic diseases, will be on cognition. This link between dietary fat intake
55 and the risk of cognitive decline has been the focus of many review papers over the last 10-15 years⁹⁻¹¹.
56 One of the most recent reviews supports a positive association between dietary and blood n-6: n-3 ratio and
57 cognitive decline and incidence of dementia, as evaluated on 14 human studies including 7 prospective
58 studies¹². A recent meta-analysis on 11 cohort studies evaluated the association between 299 metabolites
59 and general cognitive ability and dementia. They reported that higher DHA levels in blood were associated
60 with higher cognitive function in 22,887 individuals¹³. Hence, it seems that more elevated concentration of
61 n-3 FA in the blood is associated with lower cognitive decline and perhaps lower risk of other chronic
62 diseases. However, our group showed that for older participants, plasma EPA and DHA kinetics are
63 dysregulated and this will likely lower the capacity of older adults to incorporate EPA and DHA in organs

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

72 **2. Lipid and fatty acid metabolism differences during aging**

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

77 **2.1. Normal transport of fatty acids from dietary intake to their circulation in the blood:**

78 In Western adults, the diet is composed of 30 to 40% of lipids, of which 92 to 96% are long chain
79 fatty acid esterified to a glycerol thus constituting what is the main form of dietary lipid: triglycerides
80 (TG) ¹⁴. Whole-body homeostasis requires fine-tuning of fatty acid transport and utilization by
81 metabolically active tissues ¹⁵. Because of their regulatory roles in cellular fatty acid uptake and
82 utilization, membrane apolipoprotein receptors and fatty acid transporters form an integral part of this
83 homeostatic system. As a result, imbalances in lipid metabolism likely will influence the functioning
84 of fatty acid transporters and their protein levels. Lipids are not soluble in water and necessitate
85 incorporation into amphiphilic molecules called lipoproteins to circulate in the blood. Hence,
86 following ingestion of TG, they will be hydrolysed at their ester bonds by gastric and pancreatic
87 lipases into two non-esterified fatty acids (NEFA) and one monoacylglycerol (MAG) with the fatty
88 acid being in the *Sn*-2 position ¹⁶. Both forms of lipids are passively transported into enterocytes ¹⁷ via
89 diffusion or transporters such as "Fatty Acid Transport Proteins" (FATPs) and "Cluster of
90 Differentiation 36" (CD36) ¹⁸. Dietary lipids are efficiently digested and absorbed by the enterocytes
91 ¹⁹.

92 Once inside the intestinal cells, enzymes convert the NEFAs and MAG back into TG ²⁰. These
93 will be integrated in chylomicrons and exported to the lymphatic system via the Golgi apparatus ²¹.

94 The chylomicrons, now rich in exogenous triglycerides, join the bloodstream via the thoracic duct and
95 get transported to the peripheral tissues such as muscle and fat cells. In the bloodstream, lipoprotein
96 lipase (LPL) gets activated when it detects an apolipoprotein C II (apoC-II)²² on the surface of the
97 chylomicrons. The role of lipoprotein lipase is to hydrolyse the ester bonds of TGs in chylomicrons²²
98 to release NEFAs into the bloodstream where there will be an uptake by nearby cells. The loss of TGs
99 will result in a decrease in size of chylomicrons and leave chylomicrons constituents available for the
100 synthesis of native HDL disks²³. Remnant chylomicrons rich in cholesteryl esters will be captured by
101 endocytosis by hepatocytes receptors such as LDL receptor (LDLR)²² and LDL receptor-related
102 protein (LRP) ([https://onlinelibrary-wiley-
com.ezproxy.usherbrooke.ca/doi/abs/10.1002/%28SICI%291096-
9136%28199708%2914%3A3%2B%3CS75%3A%3AAID-DIA449%3E3.0.CO%3B2-9](https://onlinelibrary-wiley-com.ezproxy.usherbrooke.ca/doi/abs/10.1002/%28SICI%291096-9136%28199708%2914%3A3%2B%3CS75%3A%3AAID-DIA449%3E3.0.CO%3B2-9)). The liver can then use
104 the endogenous TG and cholesteryl esters to form the very low density lipoprotein (VLDL)²⁴. These
105 lipoproteins will be directed to peripheral tissues. Following a loss of TG, there will be a decrease in
106 VLDL density²⁵. With the action of lipoprotein lipase, VLDL will then become intermediate density
107 lipoprotein (IDL). With the action of hepatic lipase²⁵ IDL becomes low density lipoprotein (LDL).
108 LDLs carry cholesterol to tissues²⁶. LDL will be captured by their receptor, LDLR which are found
109 on cell membranes, where it will be eliminated from the bloodstream by endocytosis²⁶. LDL
110 cholesterol will be recovered in the cell. An excess of cholesterol in the tissues will cause an inhibition
111 of transcription of the genes responsible for the formation of the LDLR²⁷. It thus reduces the uptake
112 of LDL by the cells and these LDLs will remain in circulation. The remaining LDLs in the circulation
113 are more likely to be oxidized²⁸ which will thereafter contribute to the development of atherosclerotic
114 plaque²⁸.

116 2.2 Lipoprotein metabolism modification during aging

117 During aging, the metabolism of lipids is modified and causes an increase of plasma lipids. For instance,
118 the fasting plasma levels of VLDL, TG, LDL and cholesterol²⁹ are significantly higher in the elderly³⁰.
119 Higher levels of lipid and cholesterol can be the source of many health problems such as cardiovascular
120 disease and diabetes (REF =<http://diabetes.diabetesjournals.org/content/46/8/1354.full-text.pdf> +
121 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4587882/>).

122 These plasma lipid changes in the elderly can cause an increase in plasma free fatty acid levels ([https://eds-
b.ebscohost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-](https://eds-b.ebscohost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-)

124 [b113-b378e62a44c1%40sessionmgr103](http://diabetes.diabetesjournals.org/content/diabetes/37/6/667.full.pdf)). Increasing plasma FFA may result in increased plasma glucose
125 by decreasing glucose uptake into the cells. The enzymes responsible for the oxidative cascade of GLA are
126 intimately related to that of glycolysis. Thus, increased lipid oxidation inhibits glucose metabolism,
127 decreases glucose uptake in cells, and impairs glycogen storage
128 <http://diabetes.diabetesjournals.org/content/diabetes/37/6/667.full.pdf>. This promotes hyperinsulinemia
129 and ultimate insulin resistance [https://eds-b-ebshost-](https://eds-b-ebshost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-b113-b378e62a44c1%40sessionmgr103)
130 [com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-b113-](https://eds-b-ebshost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-b113-b378e62a44c1%40sessionmgr103)
131 [b378e62a44c1%40sessionmgr103](https://eds-b-ebshost-com.ezproxy.usherbrooke.ca/eds/pdfviewer/pdfviewer?vid=1&sid=3f462f39-8acd-4cbf-b113-b378e62a44c1%40sessionmgr103).

132 Insulin resistance, often seen in the elderly (<https://www.jci.org/articles/view/110908/pdf>), will also cause
133 an increase in VLDL and blood triglycerides. Insulin resistance impairs the metabolism of chylomicrons,
134 VLDL, LDL and HDL ³¹ since a lack of insulin or a lower sensitivity to insulin will reduce the catabolism
135 of chylomicrons and VLDL by LPL. During aging, there is also a higher level of LDL which remains
136 transient for a longer period of time in the plasma (Einarsson K, Nilsell K, Leijd B, Angelin B. Influence of
137 age on secretion of cholesterol and synthesis of bile acids by the liver. *N Engl J Med* 1985;313(5):277-82.
138 doi: 10.1056/NEJM198508013130501.) as a reference of this statement. = REF 30). In the long term, these
139 LDLs are more likely to be oxidized ³¹. The higher concentration of VLDL and chylomicrons in addition to
140 oxidized LDL accumulation in older insulin-resistant individuals would increase the risk of developing
141 cardiovascular disease (CVD) ³². Furthermore, the increase of LDL may be due to the diminution of bile
142 synthesis from cholesterol by the liver during aging ^{30,33}. The decrease in bile acid synthesis is due to the
143 decrease in the expression of "cholesterol 7-alpha hydroxylase" (CYP7A1) during aging. This cytochrome
144 is one of the CYP450 and regulates the formation of bile acids ³⁴. This causes a decrease in the use of
145 cholesterol by the liver as well as a reduction in LDLR expression with age. Thus, plasma LDL will have
146 lower clearance with age resulting in an increase in plasma LDL concentration in the elderly ²⁹. In the end,
147 it is possible that deregulation of LDL in the elderly is due to several different phenomena stemming from
148 the large amount of change that occurs with age. The decrease in LDL in the elderly has shown a reduction
149 in the incidence of CVD ³⁵. In particular, a study showed that long chain polyunsaturated fatty acids
150 (PUFAs) allowed an increase in LDLR expression ³⁶, which could increase the clearance rate of plasma
151 LDL in the elderly and reduce the incidence of CVD. These are some of the modification of the lipid
152 metabolism occurring during aging. Overall, there are usually higher TG and LDL levels in the blood of
153 older adults and it is important to consider these modifications in the prevention of chronic diseases but also
154 when interpreting results pertaining to fatty acid metabolism.

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2.3 Omega-3 fatty acid metabolism during aging

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

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2.3.1 Without an omega-3 fatty acid supplementation

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To our knowledge, there are ~ 24 studies that have reported the level of omega-3 fatty acids or the omega-3 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). Among the 24 studies, 7 studies reported the omega-3 index only and showed that it was higher in older participants³⁷⁻⁴³. Two studies on the omega-3 index reported an increase of about 5-7% of the omega-3 index every decade^{37, 41}. Eleven studies reported the fatty acid profile in red blood cells (RBC)^{37, 38, 41-50}. 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 omega-3 were on average 8-10 years older than those with the lowest omega-3 fatty acid levels in erythrocytes^{43, 44}. Other studies reported the level of increase in omega-3 fatty acids for each increasing decade. Hence, it is difficult to draw a clear conclusion for the omega-3 fatty acid results in RBC but it appears that at older ages, there is more omega-3 in RBC. 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 acids⁵¹, hence limiting comparisons between studies. With respect to plasma/serum PL, there were eight studies^{45, 52-58}. Six of these studies reported on average a 1.5 fold higher level of DHA in the plasma PL of older participants, aged between 50-88 years old compared with younger participants, aged between 20-49 years old^{45, 53-57}. One study reported a 2 fold higher level of EPA in plasma PL but there were no difference between ages for DHA⁵². Yet another study 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 adults⁵⁸. Overall, there is generally good evidence supporting the idea that during aging, the relative % of omega-3 fatty acids or its concentration in RBC and plasma/serum are

185 higher in the oldest participants compared to the youngest. Some of the proposed mechanism includes
186 a reduction of omega-3 fatty acids in cell membranes, higher intestinal absorption during aging, higher
187 availability and release of adipose tissue stocks. Hence, the exact mechanism behind this higher level
188 of blood omega-3 in older individuals might be multi-level but the important point here is that they
189 might be associated to longevity.

190 **2.3.2 With an omega-3 fatty acid supplementation**

191 To our knowledge, there are nine published studies specifically addressing EPA and DHA responses to an
192 omega-3 fatty acid supplement with participants of different ages (Table 2). Supplementation doses range
193 from 300 mg/d to more than 4 g/d and lasted between 6 weeks and twelve months. Seven studies evaluated
194 the fatty acid profile in the plasma whereas one study evaluated the fatty acid profile in erythrocytes only⁵⁹
195 and another did so in platelets and adipose tissues only⁶⁰. One study reported the omega-3 index pre- and
196 post-supplementation⁵⁹ and showed that a low omega-3 index at baseline and an older age predicted those
197 with a greater increase of the omega-3 index after supplementation⁵⁹. This study had similar results to
198 Vandal et al.,⁶¹ which showed that the oldest had a higher increase in DHA compared to the youngest after
199 the supplementation, but in their study, Vandal had similar DHA levels in young and old participants at
200 baseline.

201 The other studies investigated the plasma level of omega-3 fatty acids. One study reported that older
202 participants had higher omega-3 levels at baseline but after the supplementation, the increase was similar in
203 both groups⁶². The six other studies reported a higher increase in EPA⁶³⁻⁶⁷ and/or DHA⁶⁸ in older
204 participants compared to younger. The exact mechanism explaining this effect is unclear. Most of the studies
205 reported that it is unlikely that the age-related differences in EPA and DHA at baseline are due to differences
206 in intake of omega-3 PUFA with age. Rather it seems to be related to age differences in endogenous
207 production and incorporation of EPA and DHA due to hormones and hormone sensitivity, body
208 composition, and physical activity, all of which change with age⁶⁷. The study of Walker et al. also showed
209 that the adipose tissue stores less DHA with age in response to EPA + DHA supplementation, hence
210 suggesting that age-related differences in the handling and storage of exogenous supplied DHA may be
211 related to impaired insulin sensitivity with aging or to differences in body composition with aging⁶⁷. The
212 adipose tissue represents a significant store of EPA and DHA, containing the equivalent of several hundred
213 days of the fatty acid content of a typical diet. Altogether, these results support that providing a supplement
214 of omega-3 fatty acid to older adults increases their blood levels when compared to younger individuals.
215 These results may be caused by the fact that older individuals have shown to be more compliant to treatments

216 than younger people (REF = <https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-2710.2000.00315.x>),
217 causing a higher level of omega-3 in their blood. But despite that fact, those results brings into question
218 whether this type of supplementation is useful to them in the prevention of chronic diseases since they may
219 not be able to use it. Another important point is that it might also be due to their lower turnover of circulating
220 TG, hence contributing to their higher omega-3 levels, since omega-3 fatty acid levels are esterified in TG.
221 To answer some of these questions, employing ^{13}C -fatty acids is useful.

222 2.3.3 Using ^{13}C -fatty acid to evaluate their kinetics during aging

223 Tracing metabolism of ^{13}C -fatty acids may provide some insight into possible age-related changes in
224 fatty acid metabolism in humans. Metabolism of ^{13}C -DHA has been investigated in humans ⁶⁹⁻⁷¹. In
225 young adults given an oral dose of 250-280 mg ^{13}C -DHA, ^{13}C enrichment peaked at 2 h post-dose in
226 plasma triglycerides when the tracer was given in the triglyceride form, but at 6 h post-dose when the
227 tracer was esterified to phosphatidylcholine ^{69,71}. Brossard *et al.* have reported a 1.4% apparent retro-
228 conversion of ^{13}C -DHA to ^{13}C -docosapentaenoate (22:5 omega-3) and ^{13}C -EPA 3 d after giving the
229 tracer ⁷⁰. These first results showed the feasibility of tracing DHA metabolism in humans. However,
230 neither the impact of aging on ^{13}C -DHA metabolism nor its β -oxidation were investigated, although
231 both may influence the somewhat higher blood levels of EPA and DHA commonly seen in healthy
232 elderly ^{54, 65, 66, 68, 72}. Our group are pioneers in this field as we investigated the kinetics of ^{13}C -DHA
233 in six young and six elderly participants ⁷³. We found that, in the elderly, ^{13}C -DHA was 4 times higher
234 in plasma triglycerides and NEFA at 4 h post-dose, β -oxidation was 1.9 times higher, whereas
235 apparent retro-conversion of ^{13}C -DHA to other ^{13}C -omega-3 fatty acids was 2.1 times higher 24 h and
236 7 d after tracer intake compared to the young adults ⁷³. Hence, because DHA seems to remain
237 transiently for longer periods of time in the blood of the elderly compared to the young, it may thus
238 indicate that efficiency to remove DHA from the blood is lower in the elderly than in the young,
239 resulting in lower incorporation of DHA in the membrane of cells that serve to initiate signalization
240 ^{65, 66, 68, 72}. This result is consistent with the transient slower metabolism of TG and LDL in older as
241 compared to young adults and this was described in a previous section.

242 Our most recent work with tracers between old and young men was conducted with ^{13}C -EPA or
243 arachidonic acid (^{13}C -ARA), two key fatty acids that are precursors of anti- and pro-inflammatory
244 cytokines, respectively. Surprisingly, the kinetics of ^{13}C -EPA and ^{13}C -ARA was quite similar between
245 young and old men despite a time x age interaction for ^{13}C -EPA kinetics where the postprandial shape

246 of the curve was steeper in old vs young men ⁷⁴. One intriguing result we obtained was that in old
247 men, synthesis of DHA from EPA started 2 h after tracer intake whereas it was delayed to 1 d in young
248 men. This result suggests that old adults might need more DHA than what was actually provided in
249 their diet compared to the young men. However, newly synthesized DHA accumulated in the plasma
250 of old men for 7 d and this might be because it remains for a longer period in the plasma as suggested
251 by our previous study with ¹³C-DHA. Therefore, there might be a defect in old adults to uptake DHA
252 in the tissues. We also calculated that plasma half-life of ¹³C-EPA was 2 d whereas that of ¹³C-ARA
253 was 4 d, similar to that of DHA. DHA and ARA are the two most concentrated long chain
254 polyunsaturated fatty acids in brain membranes. With our β -oxidation measures using breath samples,
255 we calculated ¹³C-EPA whole-body half-life to be ~14 days in old men whereas in the younger group
256 it was ~21 days ⁷⁴. This result indicates that older adults turn over EPA ~7 days faster than the younger
257 adults. This is an intriguing result since epidemiological studies and results we obtained in previous
258 studies ^{62, 65} support that old adults have twice as much plasma EPA, hence one would anticipate a
259 lower whole-body turnover in old vs young adults. Therefore, it seems that there is somehow a
260 disconnect between plasma levels of EPA and perhaps DHA and their kinetics, thus more studies are
261 needed to understand the mechanism of these modifications and their possible consequences such as
262 potential higher risk of cognitive decline.

263 **3 Brain modifications during aging:**

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

271 **3.1. Morphological modifications of the brain during aging**

272 There are a number of morphological modifications of the brain that occur during aging. Several
273 studies have indicated that brain volume decreases over the course of the human lifespan. A review
274 conducted by Hedman et al. ⁷⁵ compiled the results of 56 longitudinal magnetic resonance imaging (MRI)
275 studies on whole brain volumes in healthy individuals and concluded that the rate of total brain volume loss

276 is not constant throughout aging. For instance, the rate of brain volume loss after 35 years of age is
277 approximately 0.2% per year. Between 35 and 60 years of age, the volume loss rate slowly increases to
278 0.5% followed by a steady volume loss of over 0.5% per year over 60 years of age⁷⁵. Furthermore, other
279 studies have indicated that volume loss in the whole brain is greater in males than in females^{76,77}.

280 Several studies demonstrate a reduction of gray matter volume during aging⁷⁸⁻⁸⁴. More specifically,
281 the volume of gray matter in the cortex and the cerebellum of older individuals is 18% and 13% smaller,
282 respectively, than those of their younger counterparts⁸¹. There is also a significant loss of gray matter in the
283 frontal, limbic, temporal, and parietal lobes but not in the occipital lobe^{78,83}. Similarly, studies have also
284 indicated that there is a decrease of white matter volume in the brains of older individuals^{81,85-87}. According
285 to Jäncke et al.⁸¹, there is a decrease in white matter volume in the cortex and cerebellum of older individuals
286 by 5% and ~9%, respectively, compared to younger adults. Moreover, one study indicated that the rate of
287 decrease of white matter is not constant during aging⁸⁷. Instead, white matter volume slowly increases
288 before the age of 40, peaks at approximately 50 years of age, and then quickly decreases after the age of 60
289⁸⁷. As well, white matter hyperintensity lesions increase in size with age in the frontal, temporal, and parietal
290 lobes but not in the occipital lobes⁸⁶.

291 In addition to age-related changes in the volume of the whole brain, gray matter, and white matter, there are
292 also differences in the volume of specific brain structures. There seems to be a general decrease in the
293 volume of the following brain structures in older individuals compared to younger individuals: cerebral
294 hemisphere⁷⁶, frontal lobe^{88,89}, parietal lobe^{77,88,89}, temporal lobe^{88,89}, thalamus^{81,90}, basal ganglia⁸⁹,
295 and the cerebellum⁸⁹. Notably, there is atrophy of the hippocampus during aging^{77,81,91-93}. A meta-analysis
296 by Fraser et al.⁹³ detailed hippocampal atrophy rates according to 28 studies. They determined that the
297 overall rate of atrophy for the entire sample was 0.85% per year⁹³. However, the rate of hippocampal
298 atrophy reported in the studies differed based on mean age of the participants: rate of atrophy was 0.38%
299 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
300 year for a mean age of greater than 70 years. In contrast to the aforementioned structures, the ventricles of
301 the brain increase in volume during aging^{76,91}. Altogether, there is generally good evidence supporting loss
302 of matter in many brain structures, including loss in white and gray matter. These losses of brain matter can
303 contribute to lower cognitive functions during aging.

304 **3.2 Modification of brain functions during aging**

305 In addition to the many structural changes that occur during aging, brain functions are also modified during
306 this period. For instance, there is an age-related decrease in glucose metabolism in the whole brain and the
307 frontal, parietal, and temporal lobes as well as in Broca's and Wernicke's areas ⁷⁷. It also seems that brain
308 activation during the execution of motor functions is modified in older adults. For example, there is a
309 decrease in blood-oxygen level dependent (BOLD) signals in multiple brain regions (sensorimotor cortex,
310 cerebellum and thalamus) of older adults during mastication and an increase in BOLD signal in the
311 prefrontal area ⁹⁴. Another study showed that classical motor coordination regions were activated during
312 complex inter-limb coordination tasks, but that there was also increased activation of higher-level
313 sensorimotor and frontal regions in older individuals ⁹⁵. Similarly, other studies have demonstrated that the
314 performance of motor tasks result in increased activation of additional brain areas such as the basal ganglia,
315 prefrontal cortex, precuneus, and the cerebellum ⁹⁶⁻⁹⁸ in older individuals.

316 Moreover, cognitive functions are modified as a result of changes in the volume of various brain structures.
317 For instance, a meta-analysis of 57 publications from the years 1984 to 1998 concluded that white matter
318 hyperintensities are linked with poorer performance on cognitive tests for processing speed, immediate and
319 delayed memory, executive functions, and global cognitive functioning ⁹⁹. Further, a decrease in the
320 thalamus volume in older individuals is associated with attenuated performance on tests assessing cognitive
321 speed ⁹⁰. An additional meta-analysis of 33 studies concluded that larger prefrontal cortex volume and
322 thickness is correlated with better executive functioning ¹⁰⁰. In regard to hippocampus volume and memory,
323 Van Petten ¹⁰¹ reported in a meta-analysis of 33 studies that the positive correlation between hippocampus
324 size and episodic memory in older adults was weaker than expected. However, a more recent study
325 demonstrated that smaller hippocampus size is significantly associated with lower performance in episodic
326 memory, working memory, processing speed, and executive function tasks ¹⁰². Similarly to motor function,
327 it has been shown that older adults recruit additional brain regions during memory tasks ¹⁰³⁻¹⁰⁵.

328 Aging is also associated with changes in the activity of brain structures involved in sensation and perception.
329 For instance, there are less areas activated in older versus younger adults in response to various odors ¹⁰⁶. A
330 meta-analysis of 105 studies concluded that the activation of the fusiform gyrus, cerebellum, and
331 hippocampus is elevated in elderly versus younger individuals during the processing of emotional faces ¹⁰⁷.
332 Moreover, older individuals had greater activation of the prefrontal cortex during more difficult perceptual
333 tasks compared to younger individuals ¹⁰⁸. The brains of older adults are also less responsive to blue light
334 stimulation compared to younger adults ¹⁰⁹.

335 More recent studies have shed light on the changes that occur in the functional neural networks of the brain.
336 It seems that aging is associated with weaker connectivity in long-range connections and stronger
337 connectivity of short-range connections ^{110, 111}. Elderly individuals also have less intra-network and greater
338 inter-network connectivity ^{112, 113}. More specifically, older individuals have less connectivity within the
339 default mode network (DMN) and somatomotor network ¹¹³, as well as greater connectivity between the
340 salience network and the executive control network (ECN) and the DMN ¹¹². Moreover, age seems to shift
341 dynamic functional connectivity from posterior to anterior regions, which is also reflected in the decreased
342 activation of posterior regions during the decline of episodic memory in older individuals ¹¹⁴.

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

346 **3.3 Modifications of brain content during aging**

347 The number of studies, particularly those that use neuroimaging techniques, that have evaluated the change
348 in human brain content during aging is limited. Post-mortem examinations of the human brain have
349 indicated that there is a change in protein and lipid content during aging. With regard to protein, one study
350 indicated that there is a 5-15% decrease in total protein content of the brain between 30 and 90 years of age
351 ¹¹⁵. A decrease in protein content in the substantia nigra, hippocampus, caudate nucleus, and gray matter
352 has also been reported ^{116, 117}. However, Söderberg et al. ¹¹⁶ found that protein content remained unchanged
353 in the cerebellum, pons, and medulla oblongata of older individuals. Similarly, a number of post-mortem
354 studies have demonstrated changes in the lipid content of older brains. For instance, Svenerholm et al. ¹¹⁸
355 reported that there is a linear decrease in cholesterol and phospholipids in the frontal and temporal cortices
356 and a curvilinear decrease in cholesterol, PLs, cerebrosides, and sulfatides in frontal and temporal white
357 matter between the ages of 20 and 100. In terms of PLs, Söderberg et al. ¹¹⁶ found that they were relatively
358 unchanged during aging with only a 5-10% decrease in the oldest age group. A more recent study conducted
359 by Hancock et al. ¹¹⁹ reported that PL content in the entorhinal cortex of older individuals is relatively stable
360 during aging, but there is an increase in mitochondrial phosphatidylcholine (PC) and a decrease in
361 mitochondrial phosphatidylethanolamine (PE). The same group reported that age is associated with an
362 increase in mitochondrial PE containing DHA, but said the increase is not large enough to increase total
363 DHA in the mitochondria. Norris et al. ¹²⁰ examined phospholipid composition in the dorsolateral prefrontal
364 cortex in individuals aged 20-100 years. They found that there is a general age-related increase in
365 phospholipids containing DHA and decrease in PLs containing ARA and docosatetraenoic acid ¹²⁰.

366 A recent study used positron emission tomography to assess the incorporation of DHA from plasma to the
367 brain using carbon-11 ([1-C¹¹])-DHA in apolipoprotein E epsilon 4 allele (APOE4) carriers versus non-
368 carriers¹²¹. APOE4 is the most important genetic risk of late-onset Alzheimer's disease¹²². Yassine et al.
369 found that the mean global gray matter DHA incorporation coefficient was 16% higher in APOE4 carriers
370 vs non-carriers¹²¹. A higher DHA incorporation coefficient was also observed in other regions including
371 the entorhinal cortex¹²¹. However, the whole-brain DHA incorporation rate was not significantly different
372 between APOE groups¹²¹. They also did not observe any age-related effects on DHA incorporation, but this
373 may be due to the fact that only 4 of their 23 participants were over 50 years old¹²¹. The authors
374 hypothesized that increased DHA incorporation in the brains of APOE4 carriers could be a compensatory
375 mechanism to counteract brain DHA loss¹²¹. Our group also documented that the metabolism of DHA is
376 imbalanced in APOE4 carriers¹²³⁻¹²⁶ and that they are perhaps more vulnerable to DHA deficiency¹²⁷.

377 **3.4 Does omega-3 fatty acid consumption improve brain structure and function?**

378 There are a number of studies that have examined the relationship between omega-3 fatty acid consumption
379 and brain structure and function. For instance, Gu et al.¹²⁸ evaluated the link between white matter integrity
380 and dietary nutrient intake in 239 elderly participants. They assessed white matter integrity using fractional
381 anisotropy measured by diffusion tensor imaging (DTI). They found that the nutrient pattern characterized
382 by high consumption of omega-3 and omega-6 fatty acids and vitamin E was positively correlated with
383 fractional anisotropy which corresponds to better white matter integrity¹²⁸. Another group examined the
384 relationship between dietary fish consumption and brain structural integrity in 260 cognitively normal adults
385 aged 65 years or older¹²⁹. Fish intake was measured using the National Cancer Institute Food Frequency
386 Questionnaire and the gray matter volume of various brain regions was measured with MRI¹²⁹. They found
387 that eating baked or broiled fish weekly is positively associated with higher gray matter volume in several
388 brain regions, including the hippocampus, posterior cingulate, precuneus, and the orbital frontal cortex¹²⁹.
389 Samieri and colleagues¹³⁰ evaluated the association between plasma EPA and DHA concentrations and
390 gray matter atrophy in the medial temporal lobe in 281 individuals aged 65 years or older. The authors
391 compared fatty acid plasma concentrations at baseline to the results of MRI examinations from baseline and
392 four years after baseline¹³⁰. They observed that greater levels of plasma EPA was associated with lower
393 atrophy of the gray matter of the right amygdala and the hippocampal/parahippocampal region; this same
394 association was not observed for plasma DHA levels¹³⁰, which is counterintuitive. Samieri et al.¹³⁰ also
395 found that increased amygdala gray matter atrophy was linked with more depressive symptoms and poorer
396 semantic memory performances compared to baseline. Lastly, Witte et al.¹³¹ assessed the connection

397 between fish oil supplement consumption and brain structure and function in 65 participants aged 50 to 75
398 years. Participants consumed either fish oil, which contained 2.2 grams of omega-3 fatty acids, or a placebo
399 daily for 26 weeks. Neuropsychological testing and MRI examinations were performed before and after the
400 intervention period. The investigators found that after the 26-week intervention period, the fish oil group
401 had better white matter structural integrity in selective white matter tracts in the frontal, temporal, parietal,
402 and limbic areas ¹³¹. They also observed that the fish oil group had significant increases in gray matter
403 volume in the left hippocampus, precuneus, the superior temporal, inferior parietal and postcentral gyri, and
404 in the right middle temporal gyrus ¹³¹. In terms of performance on cognitive measures, they found that the
405 fish oil group had an improvement of 26% on executive function scores compared to no improvement in the
406 placebo group ¹³¹. In addition, they found a positive correlation between verbal fluency scores and EPA
407 percentage in red blood cell membranes in the fish oil group after intervention ¹³¹. Although for many years
408 it was thought that an intake of fish throughout life protects against cognitive decline, the recent evidence
409 suggests that fish intake might not be required throughout life to improve brain structure and function.
410 Hence, starting an EPA+DHA supplementation after 50 years old might benefit older individuals with
411 respect to prevention of brain volume and function losses.

412 4 **Are we ready for updated recommendations on dietary omega-3 fatty acids intake during aging?**

413 In this review paper, we have outlined that there are many physiological modifications occurring during
414 aging with respect to lipid metabolism and brain volume and function losses and that an omega-3 fatty
415 acid intake might help to support the brain throughout aging. It is important to note that life expectancy
416 is longer, which means that older adults may live longer with their chronic diseases. A major concern
417 regarding old age is a decline in health, especially if this entails a loss of self-sufficiency and
418 independence caused by a decline in cognition. A decline in working memory appears to be one of the
419 major consequences of *normal* aging ^{132, 133}. As outlined in the previous sections, the brain undergoes
420 physiological change during aging. While age is one risk of cognitive decline, this multifactorial disease
421 is also increased by a complex interaction between both genetic and environmental risk factors ¹³⁴⁻¹³⁶.

422 We believe nutrition has a role to play in the prevention of cognitive decline but nutrition alone might
423 not be as efficient as a multidomain intervention. Recent evidence from the FINGER trials ¹³⁷ reported
424 that combining physical exercise, personalized nutritional recommendations to avoid nutrient
425 deficiencies, controlling cardiovascular risks and having cognitive stimulation prevented cognitive
426 decline. However, they recently refocus their message by showing that dietary changes initiated early in
427 the intervention was the most influential for global cognition improvement over two years of follow-up

428 ¹³⁸. Therefore, nutrition might have a key role to play in the prevention of cognitive decline. In the case
429 of the FINGER study, dietary recommendations were not focussed on the consumption of fish oil but
430 were either focused to alleviate nutritional deficiency including low blood levels of DHA. It also has to
431 be emphasized that there is currently no drug to prevent, cure or delay the progression of dementia and
432 that some pharmaceutical companies have shut down their research laboratories in this area. Therefore,
433 prevention strategies are currently the most efficient means since once the disease process has started,
434 there is no available drug for limiting its progression. However, there is one group in Canada working
435 on a nutritional strategy, a ketogenic beverage. They reported that a ketogenic beverage increases brain
436 energy metabolism in Alzheimer's patients ^{139, 140}.

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

442

443

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450 **Conflicts of Interest**

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

Reference	n, sex and age	Blood pool	Age-increasing effects	Age-increasing effects at baseline in blood pool		
				Omega-3 index	EPA	DHA
43	n=460, 299 men and 161 women, 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 were 9.8 y older vs lower quartile		
52	53 institutionalized elderly subjects (24 men and 29 women), ≥ 60 y (~79 y); 24 young healthy adults, 20 – 42 y (~29 y)	Plasma NEFA, TG, CE and PL	In plasma PL: EPA higher in elderly; DHA and DPA: appear lower in the elderly but non-significantly different	PL: 2.1 fold higher		
44	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		
37	704 outpatients (67% male), ~62 y	RBC	RBC Omega-3 Index increases with age	5.3% increase by 10 years increase		
45	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 (6.0 – 69 y)	RBC-PL	Increased DHA in RBC-PC and in RBC-PE, and increased DPA in RBC-PS and RBC-PE;	PC: 2.2 fold higher PE: 1.6 fold higher		
53	2793 New Zealanders ≥15 y (men and women)	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 0.3 mol% between 20 and 73 y PL: in both sexes, increased by 0.3 mol% between 20 and 73 y		

54	234 men and women (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
56	426 Inuits, 18 to 74 years: 179 men (~38.7 y) and 247 women (~37.8 y), n=254 in 18-39 y and n=172 in ≥40 y	Plasma PL	Concentrations of EPA, DHA and EPA + DHA increased significantly with age	2.4 fold higher in ≥40 y group compared to 18-39 y group	1.4 fold higher in ≥40 y group compared to 18-39 y group
57	1460 subjects, 18–74 years: 722 men (~40.6 y) and 738 women (~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	1.1 fold higher in 50-74 y compared to 18-34 y	1.2 fold higher in 50-74 y compared to 18-34 y
55	917 subjects, 18-74 y: 422 men (~36.0 y) and 495 women (~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 concentrations of EPA, DHA, and EPA+DHA did not vary according to sex, but there was a significant increase in the concentrations with age	2.5 fold higher in 50-74 y compared to 18-34 y	1.7 fold higher in 50-74 y compared to 18-34 y
47	992 participants (mainly men: >80%), age: early 50s to late 70s	RBC membranes	Lower levels EPA + DHA were significantly associated with younger age		
38	446 women, ~48,5 y (40–60 y)	RBC membrane	In women aged ≥50 years, EPA and DPA levels and omega-3 index were significantly higher compared to women under the age of 50 years.	4% higher in ≥50 compared to <50 y	13% higher in ≥50 y compared to <50 y
40	n= 3196, 55 % women, ~66 y (40-74 y)	RBC	RBC Omega-3 Index increases with age	5% increase every decade	

41	159 771 patients (48% males, 52% females) being evaluated by their physicians for CVD risk	RBC	Increases in EPA and DHA each decade. <u>After age 70</u> , significant decrease in EPA while DHA remain high	7% increase by decade until 70 y, stable thereafter	13% increase by decade up to 70 y, then 9% decrease by decade	6% increase by decade until 70 y, stable thereafter
39	6501 women aged 65–80, ~15 years follow-up	RBC	RBC Omega-3 Index increases with age:	Higher Omega-3 index quartile: 0.6 y older compared to lower quartile		
48	n=456, 320 men and 136 women, 18 to 70 y (~42.5 y)	RBC-PL	EPA+DHA: ~1.4 fold increase in both gender between 18-20 vs 60+ years			
141	411 Japanese (194 men and 217 women), 418 Koreans (240 men and 178 women) and 252 Mongolians (100 men and 152 women) aged 30-60 y	Plasma	EPA and DHA increase with age in Japanese and Koreans.			Japanese: 1.2 fold increase Koreans: 1.3 fold increase Mongolians: 1.1 fold <u>decrease</u>
58	75 adults admitted for elective surgery: 48 men (~58 y: 27-81 y) and 27 women (~58 y: 33-74 y)	Plasma PL, RBC-PL and AT	Positive correlation between EPA+DHA and age, in plasma and RBC-PL but not in AT			
42	163 adults, 74 men and 89 women, 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		
142	119 subjects for each population, Icelandic (59 males and 60 females) and Icelandic-Canadians	Plasma PL	Young Icelandic-Canadians had lower levels of EPA than the middle and oldest age groups		1.8 fold increase in oldest group compared to the youngest	

	(60 males and 59 females), 20-69 years				
143	54 women, 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 women than postmenopausal women. Those receiving HRT had significantly lower levels of DPA.		1.3 fold increase in postmenopausal women without HRT vs premenopausal
144	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 (%)
49	99 Icelandic women, 18 to 73 y (~45.8 y)	RBC	Proportions of total n-3 PUFA, EPA, and DHA correlated positively with age	↑	↑
145	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: 2.3 fold increase in serum and 2 fold increase in RBC

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

799 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
⁵⁹	n=115, 60 men and 55 women, 20 to 45 years	RBC	5 doses (0, 300, 600, 900, 1800 mg) 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
⁶²	24–28 participants in each age group (except as noted in the tables), young adult = 18–34 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
⁶³	15 young (22–35 y) and 10 older (51–71 y) women	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: 10-fold vs 8-fold and DHA: 2.5-fold vs 2-fold)
⁶⁴	6 young (23–33 y) and 6 older (51–68 y) women	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 women; however, after 3 mo of (n-3) fatty acid supplementation, older women had a significantly higher percentage of EPA and DHA: EPA: 10-fold vs 5-fold and DHA: 2.5-fold vs 1.6-fold
⁶⁵	10 young (5 men and 5 women, ~22 y) and 10 elderly (5 men and 5 women, ~75 y)	Plasma	EPA-enriched supplement (1.4 g/d of EPA and 0.2 g/d of DHA) for 6 wk	Before and after the EPA supplement, fasting plasma EPA was higher in the elderly (by 85% and 67% respectively)

66	Young (18-42 y; n=93) and old (53-70 y; n=62) men	Plasma and MNC PL	Placebo (corn oil) or 1.35, 2.7, or 4.05 g EPA/day for 12 wks	In both plasma and MNC PL : at baseline, EPA and DPA increase with age while after supplementation, only EPA increases in old men; at baseline, EPA, DPA and DHA respectively ~1.3, ~1.1 and ~1.4 higher in older in plasma and EPA and DHA respectively ~1.3 and ~1.25 higher in older in MNC; with High-EPA supplementation: EPA and DPA respectively ~1.6 and ~1.3 higher in plasma and EPA ~1.4 higher in MNC
61	Elderly (n=9, 5 males and 4 females, 74 y) and young (n=10, 5 males and 5 females, 24 y)	Plasma	680 mg/day of DHA and 320 mg/day of EPA for 3 weeks, followed by 2 weeks of wash-out	Higher baseline plasma EPA in elderly group; In response to the supplement, plasma DHA rose 42% more in the elderly but EPA responded similarly in both groups
67	n=193 (101 women, 92 men), 20–79 y	Plasma PC, CE, NEFA and TG; MNC; RBC; PLAT; BU; AT	EPA+DHA equivalent to 0, 1, 2 or 4 portions of oily fish per week, for 12 months	At baseline, EPA in AT and DHA in plasma TG and AT higher with increasing age; Following supplementation, EPA in plasma TAG higher with increasing age while DHA in AT smaller with increasing age
60	92 Danish women: half premenopausal (~43 y) and half 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 lower (P<0.05) in premenopausal group both in platelets and adipose tissue, except for EPA in platelets (P=0.05); After supplementation, increase in platelets and adipose tissue was, however, the same in both groups

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