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Kaur, Gunveen; Cameron-Smith, David; Garg, Manohar; Sinclair, Andrew J.
“Docosapentaenoic acid (22:5n-3): a review of its biological effects”. Published in
Progress in Lipid Research Vol. 50, Issue 1, p. 28-34 (2011).

Available from: <http://dx.doi.org/10.1016/j.plipres.2010.07.004>

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Accessed from: <http://hdl.handle.net/1959.13/1065230>

1 **Docosapentaenoic acid (22:5n-3): a review of its biological effects**

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27 **Key Words:**

28 n-3 polyunsaturated fatty acids (VLCPUFA), eicosapentaenoic acid (EPA), docosapentaenoic
29 acid (DPA), docosahexaenoic acid (DHA).

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48 **Abstract**

49 This article summarises the current knowledge available on metabolism and the biological
50 effects of n-3 docosapentaenoic acid (DPA). n-3 DPA has not been extensively studied
51 because of the limited availability of the pure compound. n-3 DPA is an elongated metabolite
52 of EPA and is an intermediary product between EPA and DHA. The literature on n-3 DPA is
53 limited, however the available data suggests it has beneficial health effects. *In vitro* n-3 DPA
54 is retro-converted back to EPA, however it does not appear to be readily metabolised to
55 DHA. *In vivo* studies have shown limited conversion of n-3 DPA to DHA, mainly in liver,
56 but in addition retro-conversion to EPA is evident in a number of tissues. n-3 DPA can be
57 metabolised by lipoxygenase, in platelets, to form 11-hydroxy-7,9,13,16,19- and 14-hydroxy-
58 7,10,12,16,19-DPA. It has also been reported that n-3 DPA is effective (more so than EPA and
59 DHA) in inhibition of aggregation in platelets obtained from rabbit blood. In addition, there is
60 evidence that n-3 DPA possesses 10-fold greater endothelial cell migration ability than EPA,
61 which is important in wound healing processes. An *in vivo* study has reported that n-3 DPA
62 reduces the fatty acid synthase and malonyl activity levels in n-3 DPA-supplemented mice
63 and these effects were stronger than the EPA-supplemented mice. Another recent *in vivo*
64 study has reported that n-3 DPA may have a role in attenuating age related decrease in spatial
65 learning and long term potentiation. However, more research remains to be done to further
66 investigate the biological effects of this n-3 VLCPUFA.

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73 **Abbreviations**

74 AA, arachidonic acid; ACC, acetyl coenzyme A; ALA, alpha linolenic acid; BAE, Bovine
75 aortic endothelial cells; ChREBP, carbohydrate response element binding protein; COX,
76 cyclooxygenase; CPT-1, carnitine palmitoyl transferase-1; DHA, docosahexaenoic acid; 17S-
77 H(p) DPA, 17S-hydro(peroxy) docosapentaenoic acid; DPA, docosapentaenoic acid; EC,
78 endothelial cells; EFA, essential fatty acid; EPA, eicosapentaenoic acid; FASn, fatty acid
79 synthase; HETE, 12-hydroxy- 5,8,10,14-eicosatetraenoic acid; HNF- α , hepatic nuclear factor-
80 α ; HTT, 5,8,10-heptadecatrienoic acid; LA, linoleic acid; LOX, lipoxygenase; L-PK, liver
81 pyruvate kinase; LT, leukotriene; LXR, liver X receptor; OHDPA, hydroxydocosapentaenoic
82 acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, prostaglandin; PPAR,
83 peroxisome proliferator-activated receptor; SREBP sterol regulatory element binding protein;
84 TAG, triacylglycerol; TNF- α , tumor necrosis factor- α ; TX, thromboxane; VEGF, vascular
85 endothelial growth factor; VLCPUFA, very long chain polyunsaturated fatty acids.

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98 **1. Introduction**

99 The realisation that brain grey matter from many different mammals was rich in n-3 long
100 chain polyunsaturated fatty acids (n-3 VLCPUFA), especially DHA was a stimulus for much
101 research on the biological role(s) of n-3 VLCPUFA (1, 2). Since then many studies have
102 been conducted to investigate the beneficial effects of n-3 VLCPUFA in neural function,
103 reducing risk the of cardiovascular events, diabetes mellitus, inhibiting growth of tumour
104 cells, modulating gene expression, anti-inflammatory activity and lipid lowering potential (3-
105 8). Most of these studies have been conducted on fish oils which typically contain all the
106 three n-3 VLCPUFA, namely eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA)
107 and docosahexaenoic acid, (DHA) (Fig 1). Many studies have addressed the unique actions of
108 EPA and DHA individually, because these two fatty acids have been available in purified
109 form. What has emerged from this research is that there are both unique as well as
110 overlapping actions. For example DHA has unique actions in promoting normal functioning
111 of brain, while both EPA and DHA have overlapping actions in lowering blood lipid levels.
112 Because pure n-3 DPA has not been readily available in quantity or at an affordable price, the
113 role(s) of n-3 DPA have not been systematically examined. To date few studies have been
114 conducted using pure or enriched n-3 DPA, yet the data available points to beneficial effects
115 of n-3 DPA. The aim of this review is to summarize this current knowledge on the biological
116 effects of n-3 DPA.

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118 **2. Synthesis and metabolism of n-3 DPA**

119 Alpha-linolenic acid (ALA) (n-3), one of the two essential fatty acids (EFA), can be
120 metabolized *in vivo* by desaturation and elongation enzymes to form a series of highly
121 unsaturated n-3 VLCPUFA. The major products of this pathway are EPA, DPA and DHA
122 (9). n-3 DPA is formed by chain elongation of EPA which is believed to be mediated by the
123 enzymes fatty acid elongase – 2 (FAE - 2) and FAE - 5 (10, 11). The conversion of n-3 DPA

124 to DHA was initially believed to be the result of the activity of $\Delta 4$ desaturase, converting
125 7,10,13,16,19-22:5 (DPA) to 4,7,10,13,16,19-22:6 (DHA). But later studies reported that
126 DPA was first elongated to 24:5n-3 which was then desaturated, by the activity of $\Delta 6$
127 desaturase, to form 24:6n-3 (12). 24:6n-3 is translocated from the endoplasmic reticulum to
128 the peroxisome where this 24 carbon fatty acid is then chain-shortened to 22:6n-3 (DHA) by
129 β -oxidation. However, in some marine algae like *Pavlova lutheri* and *Thraustochytrium* sp.,
130 the $\Delta 4$ desaturase cDNA has been sequenced and isolated (13, 14). It has been shown that
131 introduction of this $\Delta 4$ desaturase into *Saccharomyces cerevisiae* and *Brassica juncea* results
132 in production of DHA in vegetative tissues (13).

133 ALA supplementation studies conducted in 1960s, in rats, showed the increase in the tissue
134 proportions (liver and heart) of ALA, EPA, DPA and DHA. These were long-term studies,
135 conducted for a duration of 80-100 days, and involved refeeding rats which had initially been
136 made EFA deficient. The results showed that supplementation with ALA there were increases
137 in ALA, EPA, n-3 DPA and DHA as the dietary ALA level was increased (15-17). However,
138 most human supplementation studies have led to the belief that the major products of ALA
139 metabolism are EPA and n-3 DPA and that the capacity of humans to convert ALA to DHA
140 is limited (18-20); tracer studies report that females have greater capacity for synthesis of
141 DHA than males (19, 20). A recent review has summarised the data from various ALA
142 supplementation studies conducted in human adults and concluded that ALA supplementation
143 generally leads to an increase in plasma EPA and n-3 DPA levels but has little or no effect on
144 DHA levels (21). In animals, ALA has been shown to be more prone to deposition in adipose
145 tissue, β -oxidation or excretion via skin rather than metabolism to DHA (22) . An alternative
146 reason for limited synthesis of DHA from ALA is the competition between 24:5n-3 and ALA
147 for the $\Delta 6$ desaturase enzyme (Fig 1) (23). In other words, when there is a high ALA level,

148 the ALA itself (or indeed LA) could inhibit metabolism of 24:5n-3 to 24:6n-3, thus limiting
149 the availability of the precursor to form DHA.

150 In case of n-3 DPA, endothelial cells supplemented with DPA show a substantial increase in
151 EPA in the cells, but there is little evidence of DHA formation. Similarly when these cells
152 were supplemented with EPA, there was a significant increase in n-3 DPA, but not DHA (24,
153 25). However, media from n-3 DPA-incubated cells contained small amounts of DHA
154 suggesting that n-3 DPA was converted to DHA and then released into the media (24). In
155 primary rat hepatocytes, it was observed that ¹⁴C-EPA was elongated to n-3 DPA linearly
156 over a 24 hour period; in turn, the n-3 DPA was elongated to 24:5n-3, however no DHA was
157 detected in these primary hepatocytes. The conversion of n-3 DPA to EPA is referred to as
158 retro-conversion. The process of retroconversion was first described in 1970 (26) for DHA,
159 and subsequent work in human fibroblasts indicated the retroconversion of both DHA and n-
160 3 DPA was likely to involve the peroxisomal acyl-CoA oxidase (Fig 1) (27, 28). It has been
161 demonstrated using fibroblasts, that cells deficient in this enzyme cannot perform the chain
162 shortening of n-3 DPA to EPA (27).

163 Two recent *in vivo* studies also provide evidence for retroconversion of n-3 DPA into EPA. A
164 study conducted in *Sprague Dawley* rats reported that n-3 DPA supplementation for 7 days
165 (oral gavage of 50 mg/day of DPA as a free fatty acid) increased n-3 DPA concentrations in
166 all tissues examined and EPA concentrations in liver, heart and skeletal muscle. However, the
167 DHA concentration was increased only in liver (29). Similarly a study conducted in
168 C57BL/KsJ db/db mice reported that after 4 weeks of supplementation with a synthetic
169 triacylglycerol containing three n-3 DPA residues (tri-DPA), the proportion of EPA was
170 increased in liver and kidney but there was no evidence of an increase in DHA in any of the
171 tissues examined (30). There is evidence of formation of DHA from n-3 DPA in the retina of
172 miniature poodle dogs which received an intravitreal injection of ¹⁴C-DPA (31).

173 **3. Isomers of DPA**

174 There is another isomer of DPA which is an n-6 fatty acid. The n-6 DPA content is low in
175 most mammalian tissues, except testes tissue (32, 33). In fish & fish oils, the n-3 isomer of
176 DPA is substantially higher than the n-6 isomer (34). An algal oil from *Schizochytrium sp.*
177 which is rich in DHA, also contains about 15 % n-6 DPA (35). The physiological behaviour
178 of n-3 and n-6 DPA differs profoundly despite only differing in the position of two double
179 bonds in the acyl chain (36). Deficiency of n-3 fatty acids in animals leads to a depletion of
180 DHA and a compensatory rise in n-6 DPA level in most tissues, especially brain and retina
181 (37, 38). Supplementation with n-6 DPA did not produce the benefits afforded by DHA for
182 spatial task performance or in other words for brain function (39). In retina, DHA is the major
183 VLCPUFA in the rod outer segment (ROS) membrane phospholipids. In n-3 PUFA
184 deficiency studies, the n-6 DPA does not completely replace DHA in
185 phosphatidylethanolamine (PE) and phosphatidylcholine (PC) species in the retina and the
186 loss of this one double bond is enough to induce functional deficits in retinal signalling
187 pathways (40). Similarly, n-6 DPA could not fully support the protective role of DHA in cell
188 survival and apoptosis in mouse neuroblastoma cells (41).

189

190 **4. Biological effects of n-3 DPA**

191 The n-3 VLCPUFA have been shown to have many beneficial biological effects. These
192 include their role in cell membrane functions, eicosanoid production and regulation of gene
193 expression. However, most of these studies have been conducted using either fish oil
194 (mixture of n-3 VLCPUFA) or pure EPA and DHA. Although there are studies which suggest
195 a positive association between dietary n-3 DPA and heart health (42, 43), there are only a
196 limited number of studies which have investigated the biological effects of pure n-3 DPA and
197 most of these studies have been conducted using either endothelial cells or platelets (Table 1).

198 A recent study reported that aged rats fed either EPA or n-3 DPA for 56 days showed
199 neuroprotective effects (44). Both EPA and n-3 DPA attenuated the age-related increases in
200 caspase 3 activity and microglial activation and the changes observed were associated with
201 restoration of long term potentiation and improved performance in spatial learning task. The
202 authors reported that both n-3 DPA and EPA reduce the age-related oxidative changes *in*
203 *vivo*.

204

205 **4.1 Effect of n-3 DPA on eicosanoid production**

206 Eicosanoids are the signalling molecules in the body that control many physiological
207 systems. Eicosanoids include prostaglandins (PG), prostacyclins, thromboxanes (TX)
208 leukotrienes (LT), lipoxins, hydroxyeicosatetraenoic acid and epoxyeicosatetraenoic acid
209 (45). Eicosanoid synthesis is induced in the body in different physiological and/or
210 pathological conditions including inflammation and cancer. They are involved in modulating
211 the intensity and duration of inflammation and immune response (46). Arachidonic acid
212 (AA), is the substrate for the production of eicosanoids, under the action of cyclooxygenase
213 (COX) and lipoxygenase (LOX) enzymes. In platelets, AA is metabolised by COX to form
214 TXA₂, 5,8,10 heptadecatrienoic acid (HHT) and by LOX to 12-hydroxy-5,8,10,14-
215 eicosatetraenoic acid (12-HETE) (47). In platelets, n-3 DPA is metabolized into 11- and 14-
216 hydroxy docosapentaenoic acids via the LOX pathway (47). When platelets were incubated
217 with n-3 DPA, along with AA, this inhibited the COX enzyme thereby reducing the TXA₂
218 and HHT production from AA. In turn, more AA was available for shunting to the LOX
219 pathway resulting in increased production of 12-HETE.

220 Platelet aggregation is an early event in the development of thrombosis and is initiated by
221 TXA₂. The results from an *ex vivo* study conducted in rabbit platelets showed that EPA, n-3
222 DPA and DHA inhibited collagen- or AA-stimulated platelet aggregation dose-dependently,

223 and that n-3 DPA was the most potent inhibitor (48). These fatty acids also suppressed TXA₂
224 formation by platelets which were exposed to collagen, thrombin or AA. In these
225 experiments, n-3 DPA was the most potent inhibitor of COX-1 activity. n-3 DPA enhanced
226 formation of 12-HETE in response to collagen or AA by intact platelets, while EPA and
227 DHA had less of an effect. These results suggest that n-3 DPA possesses potent activity for
228 interfering with the COX pathway and accelerating the LOX pathway, thus inhibiting platelet
229 aggregation most effectively. In a human whole blood *ex vivo* study, n-3 DPA was equally
230 effective as EPA and DHA in inhibiting platelet aggregation, in female subjects however, in
231 male subjects only EPA inhibited platelet aggregation. (49).

232 n-3 DPA has also been shown to reduce the prostacyclin production (by two fold) in
233 endothelial cells (EC) compared with control cells when stimulated with endogenous AA-
234 mobilizing agents such as bradykinin and calcium ionophore A23187. It was also reported
235 that prostacyclin production in cells incubated with EPA was less inhibited than in cells
236 incubated with n-3 DPA. Since the inhibition was approximately proportional to the amount
237 of EPA in cells, regardless of n-3 DPA content in the cells, this study suggested that
238 inhibition of prostacyclin by n-3 DPA was due to its retro-conversion into EPA (50).

239 EPA and DHA also act as precursors of novel pro-resolving and anti-inflammatory
240 mediators. These mediators include resolvins of the E series from EPA, resolvins of the D-
241 series or their aspirin triggered forms from DHA and LOX initiated neuroprotectins from
242 DHA (51). These n-3 VLCPUFA-derived resolvins and protectins have unique structures, are
243 biosynthesized by independent pathways in leukocytes, brain, microglial and retinal cells and
244 share anti-inflammatory actions *in vivo*. Since n-3 DPA is known to be metabolised by LOX
245 enzymes, it is speculated that n-3 DPA might also act as a precursor for production of DPA-
246 related D-series of resolvins or neuroprotectins.

247

248 **4.2 Effect of n-3 DPA on endothelial cell (EC) migration**

249 EC migration and proliferation are important processes in the control of wound-healing
250 response of blood vessels. Direct pretreatment of ECs with n-3 DPA (0.01-1.0 microgram/ml)
251 resulted in a dose-dependent increase in migration in response to fetal bovine serum.
252 Moreover, maximum stimulation of EC migration by n-3 DPA pretreatment (0.5
253 microgram/ml) was achieved at a concentration one-tenth of that required for maximal
254 stimulation by EPA pretreatment (5.0 micrograms/ml), indicating that n-3 DPA is a potent
255 stimulator of EC migration. In EC, EPA was elongated to n-3 DPA, with little DHA being
256 formed (25). These data suggest that the stimulatory effect of EPA on EC migration occurs
257 via n-3 DPA, and that n-3 DPA may act as a powerful anti-atherogenic factor (25). Another
258 study conducted in bovine aortic endothelial (BAE) cells reported that the migrating activity
259 of these cells stimulated with vascular endothelial growth factor (VEGF) was suppressed by
260 DPA pretreatment. The pretreatment of BAE cells with n-3 DPA also suppressed tube-
261 forming activity induced by VEGF, which suggests its positive role in preventing
262 angiogenesis. The effect of n-3 DPA was stronger than those of EPA and DHA. n-3 DPA
263 treatment of BAE cells also caused the suppression of VEGF receptor-2 (VEGFR-2, the
264 kinase insert domain-containing receptor) expression. These data indicate that n-3 DPA has a
265 potent inhibitory effect on angiogenesis possibly through the suppression of VEGFR-2
266 expression (5).

267

268 **4.3 n-3 VLCPUFA regulate expression of several genes and enzymes**

269 One of the roles of n-3 VLCPUFA in the body is in the regulation of gene expression.
270 Although many genes and pathways have been reported to be regulated by n-3 VLCPUFA, it
271 is the ability of these n-3 VLCPUFA to regulate genes involved in lipid oxidation and cellular
272 inflammation that highlights a unique molecular activity (Fig 2). A variety of mechanisms

273 have been proposed to account for the impact on gene expression, demonstrated both acutely
274 and chronically, following n-3 VLCPUFA exposure, including: alterations in membrane
275 composition and associated lipid signalling, eicosanoid production, oxidant stress, nuclear
276 receptor activation or covalent modification of specific transcription factors (52). The
277 discovery of Gottlicher et al (1992) of nuclear receptors capable of binding fatty acids to
278 modulate gene expression established a direct role for fatty acids at nuclear level (53). The
279 main receptors that interact with n-3 VLCPUFA to regulate gene expression are peroxisome
280 proliferator receptors (PPAR), liver X receptor (LXR) and hepatic nuclear factor - 4 α (HNF-
281 4 α) (52). In addition, n-3 VLCPUFA also regulate gene expression by interacting with the
282 transcription factors including; sterol regulatory element binding protein (SREBP) and
283 carbohydrate response element binding protein (ChREBP) (54). The important lipogenic
284 genes down-regulated by n-3 VLCPUFA are SREBP-1c, acetyl CoA carboxylase (ACC-2),
285 fatty acid synthase (FASn) and ChREBP. SREBP-1c is a hepatic gene transcription factor
286 that plays an important role in controlling transcription of genes involved in fatty acid
287 synthesis, especially in liver (55).

288 Few studies have looked at the effect of pure n-3 DPA on genes involved in fat oxidation and
289 fat synthesis. However, in hepatocytes, n-3 DPA has been shown to induce PPAR α , but EPA
290 and DHA had a stronger and more consistent effects (56). A recent study reported that n-3
291 DPA reduced the expression of lipogenic genes *in vivo*. Supplementation of mice with pure
292 n-3 DPA (in TAG form) for 4 weeks significantly reduced the hepatic enzyme activity of
293 FAS and malic enzyme (ME) in the cytosolic fraction. In this study, the mice fed with n-3
294 DPA also showed a reduction in hepatic TG levels (30). The n-3 DPA fed to these animals
295 was a synthetic tri-DPA which is not present naturally in the diet.

296 n-3 DPA has also been reported to have a positive role in reducing the expression of
297 inflammatory genes. Inflammation is an immune response to injury. However, inflammation

298 in walls of blood vessels is thought to play a role in the development of atherosclerotic
299 plaques and thus lead to cardio-vascular disease. Tumor necrosis factor (TNF- α) is a
300 prototypic pro-inflammatory cytokine and a mediator of systemic inflammation and immune
301 responses. Supplementation of L929 murine fibrosarcoma cells with EPA, n-3 DPA and
302 DHA was shown to reduce TNF-induced necrotic cell death; in contrast, preincubation with
303 oleic acid, linoleic acid or 20:3n-3 did not affect TNF-induced necrosis. The order of
304 effectiveness was DHA > n-3 DPA > / =EPA (57).

305

306 **4.4 Conclusions and future perspective**

307 These data suggest that n-3 DPA may possess some beneficial and perhaps unique properties,
308 however, more extensive research is required to investigate the biological effects of pure n-3
309 DPA *in vitro* and *in vivo* as there are still questions that remain unanswered. For example is
310 n-3 DPA an effective precursor of DHA in brain?; is it a significant a reservoir of EPA in the
311 body?; is n-3 DPA conserved from β -oxidation relative to other n-3 polyunsaturated fatty
312 acids?; does n-3 DPA have any unique/specific biological properties?

313

314 **Acknowledgments**

315 The authors would like to acknowledge the funds provided by Meat and Livestock Australia
316 (Project code: D.MHN.0022) and the Molecular and Medical Research Strategic Research
317 Centre, School of Medicine, Deakin University.

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REFERENCES

- 324
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327 1. Crawford MA, Casperd NM, Sinclair AJ. The long chain metabolites of linoleic acid
328 linolenic acids in liver and brain in herbivores and carnivores. *Comp Biochem Physiol B*.
329 1976;54(3):395-401.
- 330 2. Crawford MA, Sinclair AJ. Nutritional influences in the evolution of mammalian
331 brain. In: *lipids, malnutrition & the developing brain*. Ciba Found Symp. 1971:267-92.
- 332 3. Arita M, Yoshida M, Hong S, Tjonahen E, Glickman JN, Petasis NA, et al. Resolvin
333 E1, an endogenous lipid mediator derived from omega-3 eicosapentaenoic acid, protects
334 against 2,4,6-trinitrobenzene sulfonic acid-induced colitis. *Proc Natl Acad Sci U S A*. 2005
335 May 24;102(21):7671-6.
- 336 4. Aktas H, Halperin JA. Translational regulation of gene expression by omega-3 fatty
337 acids. *J Nutr*. 2004 Sep;134(9):2487S-91S.
- 338 5. Tsuji M, Murota SI, Morita I. Docosapentaenoic acid (22:5, n-3) suppressed tube-
339 forming activity in endothelial cells induced by vascular endothelial growth factor.
340 *Prostaglandins Leukot Essent Fatty Acids*. 2003 May;68(5):337-42.
- 341 6. Kitajka K, Puskas LG, Zvara A, Hackler L, Jr., Barcelo-Coblijn G, Yeo YK, et al. The
342 role of n-3 polyunsaturated fatty acids in brain: modulation of rat brain gene expression by
343 dietary n-3 fatty acids. *Proc Natl Acad Sci U S A*. 2002 Mar 5;99(5):2619-24.
- 344 7. Fujikawa M, Yamazaki K, Hamazaki T, Wakaki K, Koizumi F, Yano S, et al. Effect
345 of eicosapentaenoic acid ethyl ester on albuminuria in streptozotocin-induced diabetic rats. *J*
346 *Nutr Sci Vitaminol (Tokyo)*. 1994 Feb;40(1):49-61.
- 347 8. Shimizu H, Ohtani K, Tanaka Y, Sato N, Mori M, Shimomura Y. Long-term effect of
348 eicosapentaenoic acid ethyl (EPA-E) on albuminuria of non-insulin dependent diabetic
349 patients. *Diabetes Res Clin Pract*. 1995 Apr;28(1):35-40.
- 350 9. Mohrhauer H, Holman RT. Tracer Experiments to Assess Metabolic Conversions of
351 Polyunsaturated Fatty Acids. *J Am Oil Chem Soc*. 1965 Jul;42:639-43.
- 352 10. Horton JD, Shah NA, Warrington JA, Anderson NN, Park SW, Brown MS, et al.
353 Combined analysis of oligonucleotide microarray data from transgenic and knockout mice
354 identifies direct SREBP target genes. *Proc Natl Acad Sci U S A*. 2003 Oct 14;100(21):12027-
355 32.
- 356 11. Wang Y, Botolin D, Christian B, Busik J, Xu J, Jump DB. Tissue-specific, nutritional,
357 and developmental regulation of rat fatty acid elongases. *J Lipid Res*. 2005 Apr;46(4):706-15.
- 358 12. Voss A, Reinhart M, Sankarappa S, Sprecher H. The metabolism of 7,10,13,16,19-
359 docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of
360 a 4-desaturase. *J Biol Chem*. 1991 Oct 25;266(30):19995-20000.
- 361 13. Qiu X, Hong H, MacKenzie SL. Identification of a Delta 4 fatty acid desaturase from
362 *Thraustochytrium* sp. involved in the biosynthesis of docosahexanoic acid by heterologous
363 expression in *Saccharomyces cerevisiae* and *Brassica juncea*. *J Biol Chem*. 2001 Aug
364 24;276(34):31561-6.
- 365 14. Tonon T, Harvey D, Larson TR, Graham IA. Identification of a very long chain
366 polyunsaturated fatty acid Delta4-desaturase from the microalga *Pavlova lutheri*. *FEBS Lett*.
367 2003 Oct 23;553(3):440-4.
- 368 15. Mohrhauer H, Holman RT. Effect of Linolenic Acid Upon the Metabolism of Linoleic
369 Acid. *J Nutr*. 1963 Sep;81:67-74.
- 370 16. Mohrhauer H, Holman RT. The Effect of Dietary Essential Fatty Acids Upon
371 Composition of Polyunsaturated Fatty Acids in Depot Fat and Erythrocytes of the Rat. *J*
372 *Lipid Res*. 1963 Jul;4:346-50.

- 373 17. Mohrhauer H, Holman RT. The Effect of Dose Level of Essential Fatty Acids Upon
374 Fatty Acid Composition of the Rat Liver. *J Lipid Res.* 1963 Apr;4:151-9.
- 375 18. Burdge GC, Calder PC. Conversion of alpha-linolenic acid to longer-chain
376 polyunsaturated fatty acids in human adults. *Reprod Nutr Dev.* 2005 Sep-Oct;45(5):581-97.
- 377 19. Burdge GC, Jones AE, Wootton SA. Eicosapentaenoic and docosapentaenoic acids
378 are the principal products of alpha-linolenic acid metabolism in young men. *Br J Nutr.* 2002
379 Oct;88(4):355-63.
- 380 20. Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic,
381 docosapentaenoic and docosahexaenoic acids in young women. *Br J Nutr.* 2002
382 Oct;88(4):411-20.
- 383 21. Brenna JT, Salem N, Jr., Sinclair AJ, Cunnane SC. alpha-Linolenic acid
384 supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans.
385 *Prostaglandins Leukot Essent Fatty Acids.* 2009 Feb-Mar;80(2-3):85-91.
- 386 22. Fu Z, Sinclair AJ. Novel pathway of metabolism of alpha-linolenic acid in the guinea
387 pig. *Pediatr Res.* 2000 Mar;47(3):414-7.
- 388 23. Portolesi R, Powell BC, Gibson RA. Competition between 24:5n-3 and ALA for
389 Δ^6 desaturase may limit the accumulation of DHA in HepG2 cell membranes. *J Lipid*
390 *Res.* 2007 Jul;48(7):1592-8.
- 391 24. Achard F, Benistant C, Lagarde M. Interconversions and distinct metabolic fate of
392 eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in bovine aortic endothelial
393 cells. *Biochim Biophys Acta.* 1995 Apr 6;1255(3):260-6.
- 394 25. Kanayasu-Toyoda T, Morita I, Murota S. Docosapentaenoic acid (22:5, n-3), an
395 elongation metabolite of eicosapentaenoic acid (20:5, n-3), is a potent stimulator of
396 endothelial cell migration on pretreatment in vitro. *Prostaglandins Leukot Essent Fatty Acids.*
397 1996 May;54(5):319-25.
- 398 26. Stoffel W, Eker, Assad H, Sprecher H. Enzymatic studies on the mechanism of the
399 retroconversion of C22-polyenoic fatty acids to their C20-homologues. *Hoppe Seylers Z*
400 *Physiol Chem.* 1970 Dec;351(12):1545-54.
- 401 27. Christensen E, Woldseth B, Hagve TA, Poll-The BT, Wanders RJ, Sprecher H, et al.
402 Peroxisomal beta-oxidation of polyunsaturated long chain fatty acids in human fibroblasts.
403 The polyunsaturated and the saturated long chain fatty acids are retroconverted by the same
404 acyl-CoA oxidase. *Scand J Clin Lab Invest Suppl.* 1993;215:61-74.
- 405 28. Reddy JK, Hashimoto T. Peroxisomal beta-oxidation and peroxisome proliferator-
406 activated receptor alpha: an adaptive metabolic system. *Annu Rev Nutr.* 2001;21:193-230.
- 407 29. Kaur G, Begg DP, Barr D, Garg M, Cameron-Smith D, Sinclair AJ. Short-term
408 docosapentaenoic acid (22:5 n-3) supplementation increases tissue docosapentaenoic acid,
409 DHA and EPA concentrations in rats. *Br J Nutr.* Jan;103(1):32-7.
- 410 30. Gotoh N, Nagao K, Onoda S, Shirouchi B, Furuya K, Nagai T, et al. Effects of three
411 different highly purified n-3 series highly unsaturated fatty acids on lipid metabolism in
412 C57BL/KsJ-db/db mice. *J Agric Food Chem.* 2009 Nov 25;57(22):11047-54.
- 413 31. Alvarez RA, Aguirre GD, Acland GM, Anderson RE. Docosapentaenoic acid is
414 converted to docosahexaenoic acid in the retinas of normal and prcd-affected miniature
415 poodle dogs. *Invest Ophthalmol Vis Sci.* 1994 Feb;35(2):402-8.
- 416 32. Tam PS, Sawada R, Cui Y, Matsumoto A, Fujiwara Y. The metabolism and
417 distribution of docosapentaenoic acid (n-6) in the liver and testis of growing rats. *Biosci*
418 *Biotechnol Biochem.* 2008 Oct;72(10):2548-54.
- 419 33. Tam PS, Umeda-Sawada R, Yaguchi T, Akimoto K, Kiso Y, Igarashi O. The
420 metabolism and distribution of docosapentaenoic acid (n-6) in rats and rat hepatocytes.
421 *Lipids.* 2000 Jan;35(1):71-5.

- 422 34. Gundstone FD, Harwood JL, Padley FB. *The Lipid Handbook*. London: Chapman &
423 Hall; 1994.
- 424 35. Sanders TA, Gleason K, Griffin B, Miller GJ. Influence of an algal triacylglycerol
425 containing docosahexaenoic acid (22 : 6n-3) and docosapentaenoic acid (22 : 5n-6) on
426 cardiovascular risk factors in healthy men and women. *Br J Nutr*. 2006 Mar;95(3):525-31.
- 427 36. Deng Y, Almsharqi ZA, Shui G, Wenk MR, Kohlwein SD. Docosapentaenoic acid
428 (DPA) is a critical determinant of cubic membrane formation in amoeba *Chaos* mitochondria.
429 *Faseb J*. 2009 Sep;23(9):2866-71.
- 430 37. Guesnet P, Pascal G, Durand G. Effects of dietary alpha-linolenic acid deficiency
431 during pregnancy and lactation on lipid fatty acid composition of liver and serum in the rat.
432 *Reprod Nutr Dev*. 1988;28(2A):275-92.
- 433 38. Homayoun P, Durand G, Pascal G, Bourre JM. Alteration in fatty acid composition of
434 adult rat brain capillaries and choroid plexus induced by a diet deficient in n-3 fatty acids:
435 slow recovery after substitution with a nondeficient diet. *J Neurochem*. 1988 Jul;51(1):45-8.
- 436 39. Lim SY, Hoshiba J, Salem N, Jr. An extraordinary degree of structural specificity is
437 required in neural phospholipids for optimal brain function: n-6 docosapentaenoic acid
438 substitution for docosahexaenoic acid leads to a loss in spatial task performance. *J*
439 *Neurochem*. 2005 Nov;95(3):848-57.
- 440 40. Niu SL, Mitchell DC, Lim SY, Wen ZM, Kim HY, Salem N, Jr., et al. Reduced G
441 protein-coupled signaling efficiency in retinal rod outer segments in response to n-3 fatty acid
442 deficiency. *J Biol Chem*. 2004 Jul 23;279(30):31098-104.
- 443 41. Kim HY, Akbar M, Lau A. Effects of docosapentaenoic acid on neuronal apoptosis.
444 *Lipids*. 2003 Apr;38(4):453-7.
- 445 42. Oda E, Hatada K, Katoh K, Kodama M, Nakamura Y, Aizawa Y. A case-control pilot
446 study on n-3 polyunsaturated fatty acid as a negative risk factor for myocardial infarction. *Int*
447 *Heart J*. 2005 Jul;46(4):583-91.
- 448 43. Rissanen T, Voutilainen S, Nyyssonen K, Lakka TA, Salonen JT. Fish oil-derived
449 fatty acids, docosahexaenoic acid and docosapentaenoic acid, and the risk of acute coronary
450 events: the Kuopio ischaemic heart disease risk factor study. *Circulation*. 2000 Nov
451 28;102(22):2677-9.
- 452 44. Kelly L, Grehan B, Chiesa A, O'Mara S, Downer E, Sahyoun G, et al. The
453 polyunsaturated fatty acids, EPA and DPA exert a protective effect in the hippocampus of the
454 aged rat. *Neurobiology of Aging*. 2010; Article in press.
- 455 45. Tassoni D, Kaur G, Weisinger R, Sinclair A. The role of eicosanoids in the brain.
456 *Asia Pac J Clin Nutr*. 2008;17(S1):220-8.
- 457 46. Calder PC, Grimble RF. Polyunsaturated fatty acids, inflammation and immunity. *Eur*
458 *J Clin Nutr*. 2002 Aug;56 Suppl 3:S14-9.
- 459 47. Careaga MM, Sprecher H. Synthesis of two hydroxy fatty acids from 7,10,13,16,19-
460 docosapentaenoic acid by human platelets. *J Biol Chem*. 1984 Dec 10;259(23):14413-7.
- 461 48. Akiba S, Murata T, Kitatani K, Sato T. Involvement of lipoxygenase pathway in
462 docosapentaenoic acid-induced inhibition of platelet aggregation. *Biol Pharm Bull*. 2000
463 Nov;23(11):1293-7.
- 464 49. Phang M, Garg ML, Sinclair AJ. Inhibition of platelet aggregation by omega-3
465 polyunsaturated fatty acids is gender specific-Redefining platelet response to fish oils.
466 *Prostaglandins Leukot Essent Fatty Acids*. 2009 Jul;81(1):35-40.
- 467 50. Benistant C, Achard F, Ben Slama S, Lagarde M. Docosapentaenoic acid (22:5,n-3):
468 metabolism and effect on prostacyclin production in endothelial cells. *Prostaglandins Leukot*
469 *Essent Fatty Acids*. 1996 Oct;55(4):287-92.
- 470 51. Serhan CN, Chiang N. Endogenous pro-resolving and anti-inflammatory lipid
471 mediators: a new pharmacologic genus. *Br J Pharmacol*. 2007 Oct 29;153(1:S):200-15.

- 472 52. Chow CK, editor. Fatty acids in foods and their health implications. Third ed. Boca
473 Raton: CRC Press; 2008.
- 474 53. Gottlicher M, Widmark E, Li Q, Gustafsson JA. Fatty acids activate a chimera of the
475 clofibrilic acid-activated receptor and the glucocorticoid receptor. *Proc Natl Acad Sci U S A*.
476 1992 May 15;89(10):4653-7.
- 477 54. Chow CK. Fatty Acids in Foods and Their Health Implications. Third, illustrated,
478 revised ed. Chow CK, editor. Boca Raton: CRC Press; 2007.
- 479 55. Horton JD, Goldstein JL, Brown MS. SREBPs: transcriptional mediators of lipid
480 homeostasis. *Cold Spring Harb Symp Quant Biol*. 2002;67:491-8.
- 481 56. Pawar A, Jump DB. Unsaturated fatty acid regulation of peroxisome proliferator-
482 activated receptor alpha activity in rat primary hepatocytes. *J Biol Chem*. 2003 Sep
483 19;278(38):35931-9.
- 484 57. Kishida E, Tajiri M, Masuzawa Y. Docosahexaenoic acid enrichment can reduce
485 L929 cell necrosis induced by tumor necrosis factor. *Biochim Biophys Acta*. 2006
486 Apr;1761(4):454-62.
- 487 58. Williard DE, Harmon SD, Kaduce TL, Preuss M, Moore SA, Robbins ME, et al.
488 Docosahexaenoic acid synthesis from n-3 polyunsaturated fatty acids in differentiated rat
489 brain astrocytes. *J Lipid Res*. 2001 Sep;42(9):1368-76.
- 490 59. Langelier B, Alessandri JM, Perruchot MH, Guesnet P, Lavalie M. Changes of the
491 transcriptional and fatty acid profiles in response to n-3 fatty acids in SH-SY5Y
492 neuroblastoma cells. *Lipids*. 2005 Jul;40(7):719-28.
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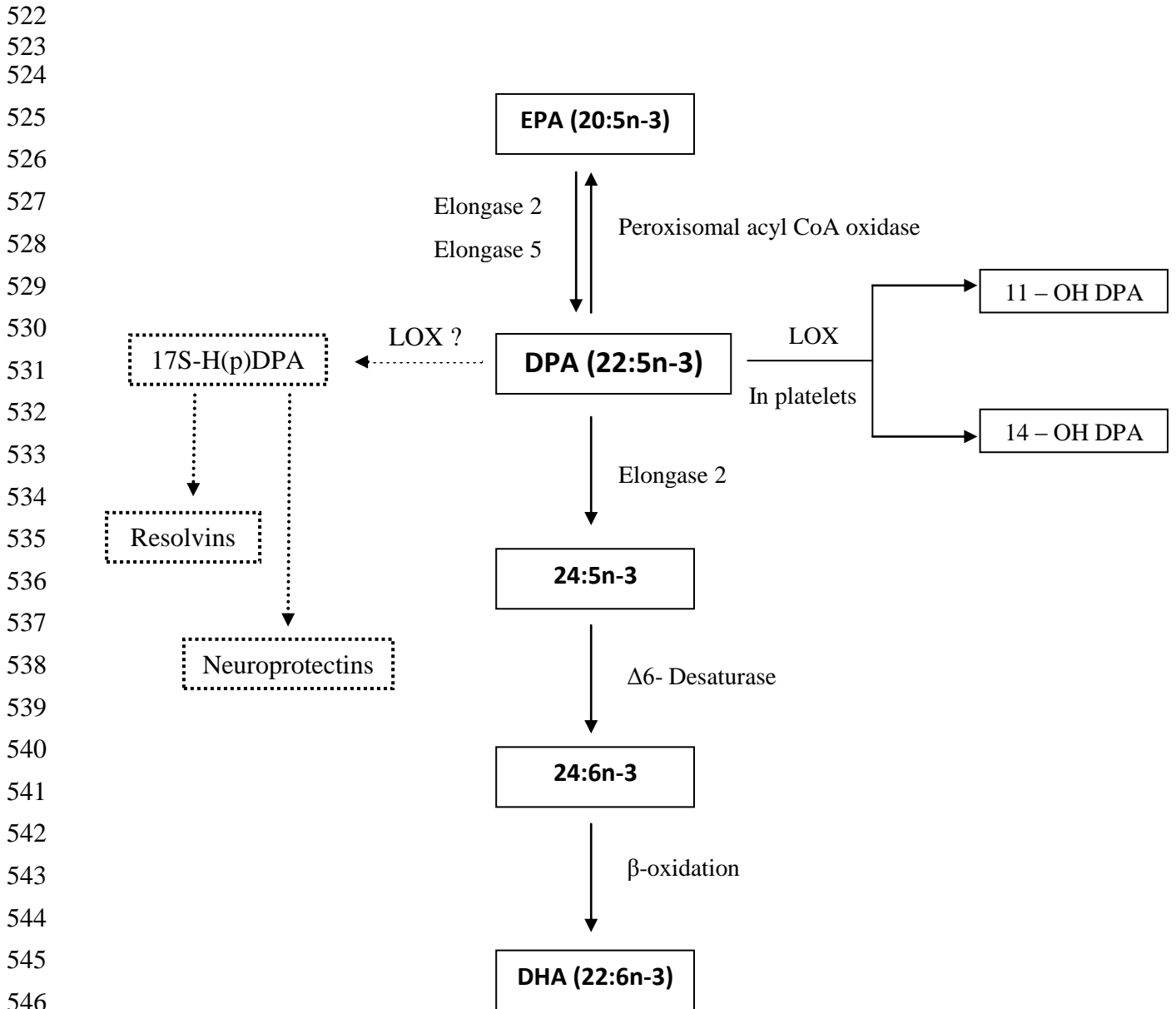
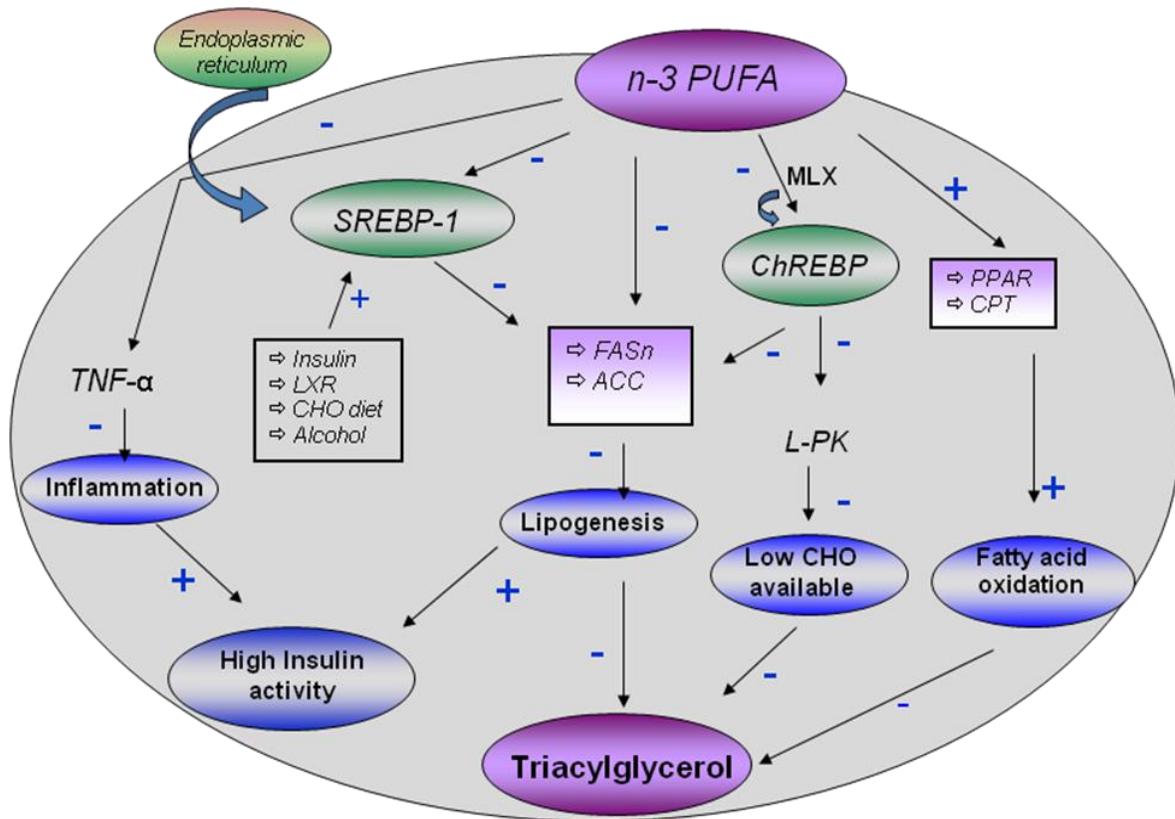


Fig 1: Metabolites of n-3 DPA. DPA forms two hydroxy acids (11- and 14-OH DPA) via an indomethacin-insensitive pathway. DPA can be retro-converted into EPA in cells and animals and is likely to involve the peroxisomal acyl coA oxidase. Since n-3 DPA is known to be metabolized by LOX enzymes, it is speculated that n-3 DPA might also act as a precursor for production of DPA-related D-series of resolvins or neuroprotectins.

(Abbreviations: EPA – Eicosapentaenoic acid; DPA – Docosapentaenoic acid; DHA – Docosahexenoic acid; LOX – Lipoxygenase; OH DPA – Hydroxy docosapentaenoic acid; 17S-H(p)DPA – 17S hydro (peroxy) docosapentaenoic acid.)



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564 **Fig 2** Mechanisms involved in triacylglycerol lowering effect of n-3 VLCPUFA. n-3
 565 VLCPUFA mediate the triacylglycerol lowering effect by upregulating fat oxidation genes
 566 like PPAR and CPT-1. They also downregulate the genes involved in fat synthesis like
 567 SREBP-1c, ACC and FASN, thereby decreasing the fat synthesis n-3 VLCPUFA also decrease
 568 expression of ChREBP which in turn lowers the expression of L-PK and lower the amount of
 569 carbohydrates available for triacylglycerol synthesis. (PUFA polyunsaturated fatty acids;
 570 PPAR peroxisome proliferator receptor ; CPT-1 carnitine palmitoyl transferase 1; SREBP-1c
 571 sterol regulatory element binding protein, L-PK liver pyruvate kinase, ACC acetyl CoA
 572 carboxylase; FASN fatty acid synthase; ChREBP carbohydrate response element binding
 573 protein.)

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582 **Table 1: List of literature available on n-3 DPA**

Year	Author	Model	Findings
<i>In vitro and ex vivo Studies</i>			
1984	Careaga and Sprecher	Human platelets	Platelets metabolize 7,10,13,16,19-DPA (22:5(n-3)) into 11-hydroxy-7,9,13,16,19- and 14-hydroxy-7,10,12,16,19-DPA via an indomethacin-insensitive pathway. n-3 DPA inhibits the synthesis of both 5,8,10-heptadecatrienoic acid and thromboxine B ₂ from arachidonic acid.
1991	Rosenthal et al	Fibroblasts and retinoblasts	Although fibroblasts desaturate [14C]22:5(n-3), the process appears to be qualitatively different from that of retinoblastoma cells.
1993	Christensen et al	Fibroblasts	Peroxisomal acyl CoA oxidase is responsible for the chain-shortening of DHA and n-3 DPA.
1995	Achard et al	Endothelial cells	EPA, n-3 DPA and DHA are actively interconverted to each other in endothelial cells.
1996	Benistant et al	Endothelial cells	n-3 DPA bound to albumin produced two-fold less prostacyclin compared to control cells when stimulated with endogenous arachidonic acid-mobilizing agents
1996	Kanayasu-Toyoda et al	Endothelial cells	The stimulative effect of EPA on EC migration occurs via n-3 DPA, and that n-3 DPA may act as a powerful anti-atherogenic factor.
2000	Akiba et al	Rabbit platelets(<i>ex vivo</i>)	EPA, n-3 DPA and DHA inhibit collagen- or arachidonic acid-stimulated platelet aggregation dose-dependently among which n-3 DPA was the most potent inhibitor.
2001	Arita et al	Human promyelocytic leukemia cells	n-3 VLCPUFA including n-3 DPA-induce apoptosis of leukemia cells (HL-60), in part by direct action on the cells and by activation of the caspase cascade through cytochrome <i>c</i> release coupled with mitochondrial membrane depolarization.
2001	Williard et al	Rat brain astrocytes	Astrocytes can synthesise and incorporate [3- ¹⁴ C]DHA into the cell PL from [3- ¹⁴ C]ALA and [3- ¹⁴ C]DPA and also release it into the media as free fatty acid (58).

2003	Tsuji et al	Endothelial cells	n-3 DPA suppressed tube-forming activity induced by vascular endothelial growth factor (VEGF) and n-3 DPA has a potent inhibitory effect on angiogenesis through the suppression of VEGFR-2 expression
2003	Pawar and Jump	Hepatocytes	Metabolic labelling indicated that a significant fraction of ¹⁴ C-EPA was elongated to n-3 DPA in hepatocytes. Cells treated with DPA or DHA led to a significant accumulation of EPA in the NEFA pool. EPA and DHA, but not n-3 DPA, are active ligands for PPAR α .
2005	Langelier et al	Neuroblastoma cells	The incorporation of EPA, DPA, and preformed DHA followed a dose-response saturating curve, whereas that of DHA synthesized either from α -LNA, EPA, or DPA peaked at concentrations of precursors below 15–30 μ M and sharply decreased with higher doses. DPA was readily formed from EPA and DHA was formed from both EPA and n-3 DPA (59).
2006	Kishida et al	Fibrosarcoma cells	Attenuation of TNF-induced necrosis by the supplementation of various C20 or C22 polyunsaturated fatty acids is mainly attributable to the enrichment of three kinds of polyunsaturated fatty acids, i.e., DHA, n-3 DPA or AA, in cell phospholipids.
2009	Phang et al	Human platelets (<i>ex vivo</i>)	EPA was significantly more effective in reducing platelet aggregation compared with n-3 DPA and DHA. However, when grouped by gender, in females all three n-3 VLCPUFA were effective. But in men EPA was more effective than n-3 DPA and DHA.
<i>In vivo</i> Studies			
1993	Alvarez et al	Miniature poodle dogs	Intravitreal injection of dogs with ¹⁴ C-DPA (n-3) led to formation of ¹⁴ C-DHA in the rod outer segment lipids. There was no difference in % dpm of DHA generated in normal dogs and dogs affected with progressive rod-cone degeneration. There was also evidence of label in 24:5 n-3 and 24:6 n-3.
2009	Kaur et al	Sprague Dawley rats	n-3 DPA can be converted to DHA in the liver, in a short-term study, and that in addition it is partly retroconverted to EPA in liver, adipose, heart and skeletal muscle.
2009	Gotoh et al	C57BL/KsJ-db/db mice	n-3 DPA and DHA treatment decreased the hepatic TG levels compared to the control while EPA was most effective in reducing serum TG levels.

2010	Kelly et al	Young and aged rats	Oral doses of n-3 DPA downregulated microglial activation and decreased the activation of sphingomyelinase and caspase 3 and consequently attenuated the age-related decrease in spatial learning and long-term potentiation.
Association Studies			
2000	Rissanen et al	-	Men in the highest fifth of the proportion of serum DHA + n-3 DPA in all fatty acids had a 44% reduced risk of acute coronary events compared with men in the lowest fifth in a prospective population study.
2005	Oda et al	-	Serum levels (% weight) of linolenic acid, EPA, n-3 DPA, and total n-3 VLCPUFA were significantly lower in patients with acute myocardial infarction than the control group in a case control study.

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584 (Abbreviations: EPA – Eicosapentaenoic acid; n-3 DPA – Docosapentaenoic acid; DHA – Docosahexaenoic acid; LOX – Lipoxygenase)

