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**Docosahexaenoic acid, protectin synthesis: relevance against athero-thrombogenesis**

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**Running title:** DHA & protectins against atherothrombosis

## **Abstract**

Docosahexaenoic acid (DHA) is an abundant nutrient from marine lipids: its specific biological effects have been investigated in human volunteers, taking into consideration the dose effects. We report herein that, at dosages below one g/day, DHA proved to be effective in lowering blood platelet function and exhibited an “antioxidant” effect. However, this was not anymore the case following 1.6g/day, showing then a U-shape response. The antioxidant effect has been observed in platelets as well as low-density lipoproteins, of which the redox status is assumed to be crucial in their relationship with atherosclerosis. Secondly, the oxygenated products of DHA, especially protectins produced by lipoxygenases, have been considered for their potential to affect blood platelets and leukocytes. It is concluded that DHA is an interesting nutrient to reduce athero-thrombogenesis, possibly through complementary mechanisms involving lipoxygenase products of DHA.

## **Key-words**

Blood platelets, Leukocytes, Cyclooxygenases, Lipoxygenases

## **Introduction**

Docosahexaenoic acid (DHA, 22:6n-3) is an abundant long-chain polyunsaturated fatty acid (PUFA) of marine origin<sup>(1)</sup>. In addition to be a major PUFA of the brain and retina in animals<sup>(2)</sup>, DHA is well-known as a relevant nutrient which prevents adverse cardiovascular events<sup>(3)</sup>. Together with its precursor eicosapentaenoic acid (EPA, 20:5n-3), another major component of marine lipids<sup>(4)</sup>, DHA is one of the two main omega-3 long-chain PUFA believed to be responsible for protection against cardiovascular events<sup>(5)</sup>. However, it is worth mentioning the possible involvement in these biological events the third most abundant component in marine lipids<sup>(6)</sup>, the intermediate between EPA and DHA, docosapentaenoic acid (DPA, 22:5n-3), and that of the omega-3 family precursor alpha-linolenic acid (ALA, 18:3n-3)<sup>(7)</sup>.

During the last decade, a series of oxygenated derivatives of DHA have been described with activities against inflammation, some speeding the resolution phase of inflammation, then playing an interesting role in the prevention of atherogenesis. The oxygenated metabolites have been named protectins and resolvins<sup>(8,9)</sup>. One protectin made by macrophages has been named maresin<sup>(10)</sup>. Lipoxygenases (LOX) are key enzymes in the generation of these derivatives, which makes the action of DHA quite specific and different from that of EPA that

is active through some of its cyclooxygenase (COX) products as well. In the eighties, DHA had already been reported as a fairly good substrate of blood platelet 12-LOX<sup>(11)</sup>. Also, its mono-hydroxylated metabolites produced through LOX have been found to be inhibitors of the thromboxane-induced platelet aggregation as well as blood vessel constriction<sup>(12)</sup>.

This short review will consider the *ex vivo* effect of dietary DHA used at low dosage, and the *in vitro* biological effects of a double lipxygenase product of DHA called protectin DX, in the frame of the cardiovascular risk.

### **DHA supplementation in humans**

A large number of trials have been conducted with long-chain n-3 PUFA with various proportions of EPA and DHA, and also DPAn-3 when more or less crude fish oil or fish meat were used for supplementing the diet. It is generally assumed that those supplementations have preventive effects against cardiovascular events. However, depending on the status of volunteers (age, dietary habits, possible usage of drugs, etc) the benefits of such supplementations may be controversial. Indeed, a recent meta-analysis which did not take into account the different situations of the people participating in the trials concluded that “omega-3 PUFA supplementation was not associated with a lower risk of all-cause mortality”<sup>(13)</sup>. A post-review further indicates that “Subgroup analyses suggested that this could be because of a low absolute risk as a consequence of the state-of-the-art drug treatment”<sup>(14)</sup>.

Earlier, we have considered that the dosage in EPA-DHA supplementation could be an issue as their high level of unsaturation makes them highly susceptible to peroxidation that could be detrimental to their potential benefit. Relating to blood platelets that play a crucial role in the initiation of atherothrombogenesis, we first considered that the increased oxidative stress associated with aging<sup>(15)</sup> could negatively affect the expected benefit of the intake of long-chain n-3 PUFA. So, we conducted an assay with a small dosage of those (150mg DHA + 30 mg EPA esterified in triglycerides), each day for six weeks. The main results at the platelet level were a significant accumulation of DHA in membrane ethanolamine phospholipids and an increase in platelet vitamin E, the latter being of interest because platelet vitamin E is lower in this population of elderly people compared to young adults, associated with a decrease in malondialdehyde (MDA) concentration<sup>(16)</sup>. Also, a trend in decreased platelet aggregation and basal thromboxane formation could be observed, but the most striking fact was a significant lowering of the diastolic blood pressure. In contrast, no differences could be

observed in a placebo group receiving the same amount of sunflower oil, which of course contained high proportion of linoleate and no n-3 PUFA<sup>(16)</sup>.

More recently we have conducted another investigation with increasing intakes of pure DHA esterified in triglycerides (algal oil) in a population of middle-aged men (53-65 year-old) with each of them following the supplementation program (two weeks of successively 200, 400, 800 and 1600 mg DHA per day), making each volunteer his own control. A significant dose-dependent accumulation of DHA could be observed in platelet phospholipids, but platelet aggregation was only significantly lowered after the intermediate dosages (400 and 800 mg/d). In terms of redox status, only the 200 mg/d dosage was able to increase platelet vitamin E. Most interesting was to find that urinary isoprostanes, a recognized marker of oxidative stress, were significantly decreased after 200mg/d and significantly increased after 1600 mg/d<sup>(17)</sup>. It must be noticed that the other markers of the oxidative stress, taken into consideration in this study, were not significantly altered following the highest dosage. Regarding the plasma low-density lipoproteins (LDL), their phospholipids and cholesteryl esters dose-dependently accumulated DHA, but significant improvements of the redox status were observed after 200, 400 and 800 mg/d DHA intake only. This concerned vitamin E with the highest increase after 200 mg/d, and reciprocal U-shape curves for MDA, a global marker of oxidative stress, and the oxidizability of LDL to copper ions, with a decreased MDA and increased lag phase of oxidation in response to copper<sup>(18)</sup>. These results clearly indicate that low daily intake of DHA (lower than 1 g/d) allows expression of an “antioxidant” profile based on several blood markers. This beneficial profile was not any more observed following the highest dosage, with even a global increased oxidative stress as stated above with urinary isoprostanes.

Overall, and although the two latter intervention studies in healthy humans have not been conducted on a long term basis, they clearly indicate that the amount of long-chain n-3 PUFA intake is an issue that must be taken into consideration.

### **Oxygenated metabolism of DHA and biological effects**

Contrary to EPA, that has the structural feature of arachidonic acid (ARA) with an additional cis/Z double bond at carbon 17, DHA is not oxygenated into prostanoid-like products by cyclooxygenases (COX) although it may inhibit the enzymes by competition, especially against ARA<sup>(19)</sup>. DHA may however be hydroxylated into 13-hydroxylated derivative by COX-2 or into 17(R)-hydroxylated derivative if COX-2 is treated by aspirin, the latter

derivative being a substrate of the neutrophil 5-LOX to produce resolvins D1 and D2<sup>(20)</sup>. This production is likely to be very low as the rate conversion of DHA into its 17(R)-hydroxylated derivative has been found to be less than 1-5 % of the rate conversion of ARA into PGH<sub>2</sub><sup>(21)</sup>. In contrast, DHA is a fairly good substrate of LOX to produce various hydroxylated end-products after reduction of the hydroperoxide intermediates by glutathione peroxidase. They are 4- and 7-OH through 5-LOX, 11- and 14-OH through 12-/n-9-LOX and 17-OH through 15-/n-6-LOX<sup>(22)</sup>. The oxygenation of DHA through the latter LOX has been studied in details for the production of protectin/neuroprotectin D1<sup>(8)</sup>. In this case the biosynthetic route seems to mimic leukotriene B<sub>4</sub> production by 5-LOX from ARA, then leading to 10(R),17(S)diOH-4Z,7Z,11E,13E,15Z,19Z-22:6<sup>(23,24)</sup>.

A double 15-/n-6-LOX end-product can also be produced, which is a geometric and stereoisomer of PD1. It is 10(S),17(S)diOH-4Z,7Z,11E,13Z,15E,19Z-22:6 and has been named protectin DX (PDX)<sup>(25)</sup>. As mono-hydroxylated derivatives have been shown to inhibit the thromboxane-induced aggregation of human blood platelets<sup>(12)</sup>, we have investigated the inhibition of that function by PDX and some isomers. In summary, PDX inhibits dose-dependently platelet aggregation induced by collagen, ARA and the stable thromboxane A<sub>2</sub> mimetic U-46619 (a stable analog of prostaglandin H<sub>2</sub>). The inhibition power of PDX towards the aggregation induced by collagen or ARA was the same, but the inhibition of the U-46619-induced aggregation was around half, suggesting that PDX did not affect the release of ARA from phospholipids in response to collagen but equally inhibits platelet COX-1 and thromboxane A<sub>2</sub> response<sup>(26)</sup>. The inhibition of COX-1 was confirmed by studying the oxygenation of radiolabelled ARA, which proved the specific inhibition of COX-1 as the 12-/n-9-LOX activity was not affected. Interestingly, a stereoisomer of PDX (10(R) instead of 10(S)), other double 15-/n-6-LOX end-products from ARA and 22:3n-6 were as inhibitory as PDX, providing they have the *E,Z,E* conjugated triene geometry. In contrast, isomers having an *E,E,Z* or *E,E,E* (all-trans) conjugated triene geometry were inactive. The *E,Z,E* conjugated trienes oxygenated PUFA have been collectively named "poxyrins"<sup>(26)</sup>. It is worth noting that PD1, which has an *E,E,Z* conjugated triene motif, is described as an anti-inflammatory molecule but as a weak inhibitor of ADP-induced aggregation, without being further potentiated by aspirin treatment<sup>(27)</sup>.

More recently, we have extended our investigations regarding PDX activities. First we found that it inhibits purified COX-1 and COX-2 with a slightly stronger inhibition of COX-2 (submitted). Second, we addressed the possibility of inhibiting the reactive oxygen species

(ROS) generation in human neutrophils, as a previous work has shown that punicic acid, which exhibits a Z,E,Z conjugated triene motif, inhibits NADPH oxidase-induced ROS production<sup>(28)</sup>. Indeed, we found such an inhibition with a dose-dependent effect<sup>(29 & submitted)</sup>. However, whereas PDX is active against platelet aggregation and COX activities in the sub-micromolar range, the inhibition of ROS generation requires micromolar concentrations. Leukotriene production from ARA being an important feature in neutrophils, we also tested the effect of PDX upon the formation of 5-LOX products in these cells. No inhibition can be found while the endogenous COX-2 activity was inhibited at similar range concentrations as those inhibiting ROS production (submitted). This indicates that in addition to its potential for inhibiting platelet function, PDX may also exhibit some anti-inflammatory activity, likely through the inhibition of COX-2.

## Conclusion

DHA is a nutrient with several beneficial effects in preventing athero-thrombogenesis if it is consumed in moderate amounts. In this respect, several international recommendations agreeing with half-a-gram per day seem reasonable to avoid some possible side-effects in terms of oxidative stress. Several mechanisms could contribute to the beneficial effects. Among them, the inhibition of COX activities and the DHA oxygenated products, mainly through LOX activities, may act by complementary effects such as inhibition of platelet aggregation and immune-competent cell function (Figure 1). Altogether the athero-thrombogenesis could be reduced. However, it remains to prove that enough oxygenated products are generated *in situ* to account for the effects observed *in vitro*.

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The authors declare no conflicts of interest.

M. Lagarde wrote the review and supervised the experimental work

M. Liu, P. Chen & F. Driss conducted experiments

E. Véricel, C. Calzada & M. Guichardant conducted and supervised the experimental work

**Figure: Summary of the DHA effect on blood platelets.**

DHA from the blood flow is partly taken up by platelets and mainly esterified into ethanolamine plasmalogens. This may be accompanied with an "antioxidant" effect as shown by *in vitro* enrichment<sup>(30)</sup>. DHA may be released by calcium-independent phospholipase A<sub>2</sub> (iPLA<sub>2</sub>)<sup>(31)</sup>, and be converted into 14-HDoHE by 12-/n-9-LOX to inhibit thromboxane action. Some non-esterified DHA entering platelets may directly be converted into 14-HDoHE. Non-esterified DHA may also inhibit thromboxane A<sub>2</sub> (TxA<sub>2</sub>) formation, in cPLA<sub>2</sub>-dependent activated platelets, through inhibition of COX-1. Besides platelets, DHA may be converted into PD1 and PDX by other cells doted of 15-/n-6-LOX such as endothelial cells and leukocytes, and PDX may inhibit platelet aggregation.

PL-ARA & PL-DHA: ARA & DHA-containing phospholipids, respectively. TxS: thromboxane synthase.

**References**

1. Ackman RG (1989) Nutritional composition of fats in seafoods. *Prog Food Nutr Sci* **13**, 161-289.
2. Bazan NG, Gordon WC & Rodriguez de Turco EB (1992) Docosahexaenoic acid uptake and metabolism in photoreceptors: retinal conservation by an efficient retinal pigment epithelial cell-mediated recycling process. *Adv Exp Med Biol.* **318**, 295-306.
3. Leaf A & Kang JX (1998) Omega 3 fatty acids and cardiovascular disease. *World Rev Nutr Diet* **83**, 24-37.
4. Weaver BJ & Holub BJ (1988) Health effects and metabolism of dietary eicosapentaenoic acid. *Prog Food Nutr Sci* **12**, 111-150.
5. Dyerberg J & Jørgensen KA (1982) Marine oils and thrombogenesis. *Prog Lipid Res* **21**, 255-269.
6. Joensen AM, Schmidt EB, Dethlefsen C *et al.* (2010) Dietary intake of total marine n-3 polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid and docosapentaenoic acid and the risk of acute coronary syndrome - a cohort study. *Br J Nutr* **103**, 602-607.
7. Mozaffarian D (2005) Does alpha-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. *Altern Ther Health Med* **11**, 24-30.



208 8. Ariel A & Serhan CN (2007) Resolvins and protectins in the termination program of acute  
209 inflammation. *Trends Immunol* **28**, 176-183.

210 9. Schwab JM, Chiang N, Arita M *et al.* (2007) Resolvin E1 and protectin D1 activate  
211 inflammation-resolution programmes. *Nature* **447**, 869-874.

212 10. Serhan CN, Yang R, Martinod K *et al.* (2009) Maresins: novel macrophage mediators  
213 with potent antiinflammatory and proresolving actions. *J Exp Med* **206**, 15-23.

214 11. Avelaño MI & Sprecher H (1983) Synthesis of hydroxy fatty acids from 4, 7, 10, 13, 16,  
215 19-[1-14C] docosahexaenoic acid by human platelets. *J Biol Chem* **258**, 9339-9343.

216 12. Croset M, Sala A, Folco G *et al.* (1988) Inhibition by lipoxygenase products of TXA<sub>2</sub>-like  
217 responses of platelets and vascular smooth muscle 14-Hydroxy from 22:6n-3 is more potent  
218 than 12-HETE. *Biochem Pharmacol* **37**, 1275-1280.

219 13. Rizos EC, Ntzani EE, Bika E *et al.* (2012) Association between omega-3 fatty  
220 acid supplementation and risk of major cardiovascular disease events: a systematic review and  
221 meta-analysis. *JAMA* **308**, 1024-1033.

222 14. Kromhout D (2012) Omega-3 fatty acids and coronary heart disease. The final verdict?  
223 *Curr Opin Lipido* **23**, 554-559.

224 15. Véricel E, Croset M, Sedivy P *et al.* (1988) Platelets and aging. I-Aggregation,  
225 arachidonate metabolism and antioxidant status. *Thromb Res* **49**, 331-342.

226 16. Véricel E, Calzada C, Chapuy P *et al.* (1999) The influence of low intake of n-3 fatty  
227 acids on platelets in elderly people. *Atherosclerosis* **147**, 187-192.

228 17. Guillot N, Caillet E, Laville M *et al.* (2009) Increasing intakes of the long-chain omega-3  
229 docosahexaenoic acid: effects on platelet functions and redox status in healthy men. *FASEB J*  
230 **23**, 2909-2916.

231 18. Calzada C, Colas R, Guillot N, *et al.* (2010) Subgram daily supplementation with  
232 docosahexaenoic acid protects low-density lipoproteins from oxidation in healthy men.  
233 *Atherosclerosis* **208**, 467-472.

234 19. Rao GH, Radha E & White JG (1983) Effect of docosahexaenoic acid (DHA) on  
235 arachidonic acid metabolism and platelet function. *Biochem Biophys Res Commun* **117**, 549-  
236 555.

237 20. Serhan CN, Hong S, Gronert K *et al.* (2002) Resolvins: a family of bioactive products of  
238 omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter  
239 proinflammation signals. *J Exp Med* **196**, 1025-1037.

21. Sharma NP, Dong L, Yuan C *et al.* (2010) Asymmetric acetylation of the cyclooxygenase-2 homodimer by aspirin and its effects on the oxygenation of arachidonic, eicosapentaenoic, and docosahexaenoic acids. *Mol Pharmacol* **77**, 979-986.
22. Lagarde M, Bernoud-Hubac N, Calzada C *et al.* (2013) Lipidomics of essential fatty acids and oxygenated metabolites. *Mol Nutr Food Res* (In the Press).
23. Mukherjee PK, Marcheselli VL, Serhan CN *et al.* (2004) Neuroprotectin D1: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. *Proc Natl Acad Sci U S A* **101**, 8491-8496.
24. Marcheselli VL, Mukherjee PK, Arita M *et al.* (2010) Neuroprotectin D1/protectin D1 stereoselective and specific binding with human retinal pigment epithelial cells and neutrophils. *Prostaglandins Leukot Essent Fatty Acids* **82**, 27-34.
25. Chen P, Fenet B, Michaud S *et al.* (2009) Full characterization of PDX, a neuroprotectin/protectin D1 isomer, which inhibits blood platelet aggregation. *FEBS Lett* **583**, 3478-3484.
26. Chen P, Véricel E, Lagarde M *et al.* (2011) Poxytins, a class of oxygenated products from polyunsaturated fatty acids, potently inhibit blood platelet aggregation. *FASEB J* **25**, 382-388.
27. Dona M, Fredman G, Schwab JM *et al.* (2008) Resolvin E1, an EPA-derived mediator in whole blood, selectively counterregulates leukocytes and platelets. *Blood* **112**, 848-855.
28. Boussetta T, Raad H, Lettéron P *et al.* (2009) Punicic acid a conjugated linolenic acid inhibits TNF $\alpha$ -induced neutrophil hyperactivation and protects from experimental colon inflammation in rats. *PLoS One* **4**, e6458.
29. Liu M, Chen P, Véricel E *et al.* (2011) Anti-aggregatory and anti-inflammatory effects of protectin DX, a major 15-lipoxygenase product of docosahexaenoic acid. *Chem Phys Lipids* **164**, 50S.
30. Véricel E, Polette A, Bacot S *et al.* (2003) Pro- and antioxidant activities of docosahexaenoic acid on human blood platelets. *J Thromb Haemost* **1**, 566-572.
31. Strokin M, Sergeeva M & Reiser G (2003) Docosahexaenoic acid and arachidonic acid release in rat brain astrocytes is mediated by two separate isoforms of phospholipase A2 and is differently regulated by cyclic AMP and Ca<sup>2+</sup>. *Br J Pharmacol* **139**, 1014-1022.

# Figure 1

