Invited commentary

A new piece in the puzzling effect of n-3 fatty acids on atherosclerosis?

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ABSTRACT

Omega-3 fatty acids (n-3) FA are reported to be protective against cardiovascular disease (CVD), notably through their beneficial action on atherosclerosis development. In this context dietary intake of long-chain marine eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) is recommended and randomised trials largely support that EPA and DHA intake is associated with a reduction of CVD. However, mechanisms governing the atheroprotective action of n-3 FA are still unclear and numerous studies using mouse models conducted so far do not allow to reach a precise view of the cellular and molecular effects of n-3 FA on atherosclerosis. In the current issue of Atherosclerosis, Chang et al. provide important new information on the anti-atherogenic properties of n-3 FA by analysing the incremental replacement of saturated FA by pure fish oil as a source of EPA and DHA in Ldlr−/− mice fed a high fat/high cholesterol diet.

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Cardiovascular disease (CVD) is the leading causes of death in the world [1] and is frequently associated with atherosclerosis, a pathology characterised by the accumulation of lipids, mainly cholesterol in the arterial wall. Among major risk factors for CVD, circulating levels of lipids and more especially those originating from diets are closely linked to development of atherosclerosis. In this context, not only cholesterol, but also dietary fatty acids (FA) may appear particularly deleterious in regards to atherosclerosis and associated CVD [2]. However, although saturated fats are proatherogenic, omega-3 fatty acids (n-3 FA), and more especially long-chain marine eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), exert atheroprotective properties through several potential underlying mechanisms [3]. Therefore, the intake of EPA and DHA is recommended around the world and randomised trials with n-3 FA confirmed that EPA and DHA intake reduced risk for CVD events [4]. However benefits of n-3 FA intake were challenged by recent clinical trials that failed to replicate protective effects of EPA + DHA on CVD, raising the controversy on the healthy side of marine n-3 FA [5].

Animal models are commonly employed in order to decipher mechanisms by which n-3 FA exert their beneficial actions regarding lipid metabolism and atherosclerosis [6]. Since the last past 20 years, mouse models, and more especially genetically modified mouse models, became the reference model to evaluate the effects of dietary fatty acids, especially n-3 FA, on atherosclerosis development [7–20]. However, the use of different mouse models of atherosclerosis (Apoe−/−, Ldlr−/−, double Apoe−/− x Ldlr−/−, Ldlr−/− x hApoB mice), as well as diet composition (chow, high cholesterol, high fat, high fat, high cholesterol/high fat), source of n-3 FA supplementation (fish oil, perilla seed oil, flaxseed, pure ALA, EPA or DHA), duration of the diet (from 4 to 32 weeks), size of atherosclerotic lesions in control animals (from 51 to 700.103 μm²) in those studies led to heterogeneous results and therefore to a partial understanding of the effects of n-3 FA on atherosclerosis. For more clarity on the main findings obtained in mouse models, those studies are summarised in Table 1.

Contrary to what observed in Apoe−/− mice, dietary supplementation of Ldlr−/− mice with n-3 FA led to a reproducible reduction of aortic atherosclerosis, although to various degrees, confirming that Ldlr−/− mice constitute the most appropriate model for studying the atheroprotective effects of n-3 FA (Table 1). When evaluated, the decrease of atherosclerosis upon n-3 FA-rich diet was accompanied by a reduction in the macrophage content as well
Table 1
Impact of dietary enrichment in n-3 fatty acids on atherosclerosis development in mouse models.

<table>
<thead>
<tr>
<th>Study</th>
<th>n-3 FA supplementation</th>
<th>Diet</th>
<th>Weeks</th>
<th>TC</th>
<th>TG</th>
<th>HDL</th>
<th>Effect of n-3 FA enrichment</th>
<th>Size in control diet</th>
<th>MΦ content</th>
<th>Inflammation</th>
<th>Major findings</th>
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</thead>
<tbody>
<tr>
<td>Brown AL et al. [7]</td>
<td>10% palm oil/0.2% chol. + 10% palm oil, echium oil (botanical 18:4 n-3), fish oil</td>
<td>6/8-week female Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>16</td>
<td>(\downarrow)</td>
<td>nd</td>
<td>nd</td>
<td>(40-70%) (aortic roots)</td>
<td>(650.10^{3},\mu m^2) (Palm oil)</td>
<td>(30-40%) (CD68)</td>
<td>nd</td>
<td>splenic/circulating Ly6Chi mononcytosis, splenic monocyte trafficking into the aortic root, splenic/circulating neutrophils, monocyte trafficking, splenic/circulating neutrophils, CD4+ T cells, mature DCs in atherosclerotic lesions, splenic immature DC (CD11c&lt;sup&gt;+&lt;/sup&gt; CD80&lt;sup&gt;−&lt;/sup&gt; CD86&lt;sup&gt;−&lt;/sup&gt;), splenic IDO DC, lesion α-SMA and collagen content, LCAT activity, hepatic apoA-I, LCAT, SR-B1, ABCA1, ABCG1, LXRα, PPARα, γ</td>
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<td>Nakajima K et al. [8]</td>
<td>8-week male Ldlr&lt;sup&gt;−/−&lt;/sup&gt; (21% fat, 1.25% chol, 0.5% cholate) followed by normal diet +/− 5% EPA</td>
<td>6-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>4</td>
<td>(\downarrow)</td>
<td>nd</td>
<td>(\downarrow)</td>
<td>(20.9%) regression ((\downarrow) + IDO inhibitor)</td>
<td>(538.10^{3},\mu m^2) (chow)</td>
<td>(23%) (MOMA-2)</td>
<td>TNFα, VCAM-1, IFN-γ, IL-10, IL-12p40, TNFα, MMP-2, MMP-9, ICAM-1, TGF-β, CD4&lt;sup&gt;+&lt;/sup&gt; T cells, mature DCs in atherosclerotic lesions, splenic immature DC (CD11c&lt;sup&gt;+&lt;/sup&gt; CD80&lt;sup&gt;−&lt;/sup&gt; CD86&lt;sup&gt;−&lt;/sup&gt;), splenic IDO DC, lesion α-SMA and collagen content, LCAT activity, hepatic apoA-I, LCAT, SR-B1, ABCA1, ABCG1, LXRα, PPARα, γ</td>
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<tr>
<td>Winnik et al. [9]</td>
<td>0.21% chol. + high (7.3%) vs low (0.03%) plant ALA content</td>
<td>8-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>16</td>
<td>(\downarrow)</td>
<td>nd</td>
<td>nd</td>
<td>(50%) (thoraco-abdominal aortae)</td>
<td>(125.10^{3},\mu m^2) (low ALA)</td>
<td>(\downarrow) (CD68)</td>
<td>TNFα, VCAM-1, CD4&lt;sup&gt;+&lt;/sup&gt; T cells in atherosclerotic lesions, circulating monocytes and lymphocytes, Shift prostaglandin and isoprostane formation towards 3-series compounds, LCAT activity, hepatic apoA-I, LCAT, SR-B1, ABCA1, ABCG1, LXRα</td>
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<td>Zhang et al. [10]</td>
<td>5% fat + safflower oil (83% LA)/perilla seed oil (81% ALA) (n-6/n-3 ratio : 1.28, 5.03, 9.98, 68.25)</td>
<td>8-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>6</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>(51.10^{3},\mu m^2) (control, no ALA)</td>
<td>nd</td>
<td>nd</td>
<td>plasma IL-6, peritoneal m&lt;sup&gt;+&lt;/sup&gt; chole, MCP-1, TNFα, ABCA1, m&lt;sup&gt;−&lt;/sup&gt;IL-6, CD36, SR-B1, MSR1, PPARα, β&lt;sub&gt;0&lt;/sub&gt;, γ, lesion α-SMA and collagen content, peritoneal m&lt;sup&gt;−&lt;/sup&gt; infiltration, aortic MMP-2, MMP-9, aortic lesion PPARα, ABCA1</td>
<td></td>
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<tr>
<td>Wang et al. [11]</td>
<td>20% fat, 0.2% chol. + 6% : EPA : DHA (no EPA + DHA, 20:1, 4:1, 1:1 ratio) (safflower oil : fish oil)</td>
<td>8-week male Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>32</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>(whole aorta)</td>
<td>nd</td>
<td>(\downarrow) (MOMA-2)</td>
<td>nd</td>
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<td>Matsumoto et al. [12]</td>
<td>0.15% chol, 15% butter +/− 5% EPA</td>
<td>5-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>13</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>(\downarrow)</td>
<td>27%/73% (aortic root/whole aorta)</td>
<td>(429.10^{3},\mu m^2) (no EPA)</td>
<td>(\downarrow) (F4/80)</td>
<td>nd</td>
<td>plasma IL-6, peritoneal m&lt;sup&gt;−&lt;/sup&gt; chole, MCP-1, TNFα, ABCA1, m&lt;sup&gt;−&lt;/sup&gt;IL-6, CD36, SR-B1, MSR1, PPARα, β&lt;sub&gt;0&lt;/sub&gt;, γ, lesion α-SMA and collagen content, peritoneal m&lt;sup&gt;−&lt;/sup&gt; infiltration, aortic MMP-2, MMP-9, aortic lesion PPARα, ABCA1</td>
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<th>Atherosclerotic lesions Effect</th>
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<td>TC</td>
<td>TG</td>
<td>HDL</td>
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<td>nd</td>
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<td>Dupasquier C. et al. [13]</td>
<td>4-week male Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>idem</td>
<td>12</td>
<td>12</td>
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<td></td>
<td>5/7-week female Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow diet +/− 10% flaxseed vs 2% chol. Diet +/− 10%, 5%, 1% flaxseed or 5% coconut oil</td>
<td>24</td>
<td>24</td>
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<tr>
<td>Saraswathi et al. [14]</td>
<td>8/12-week female Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>35% fat, 0.5% chol. + 6% olive oil or fish oil (menhaden oil)</td>
<td>12</td>
<td>12</td>
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<tr>
<td>Zampolli et al. [15]</td>
<td>5-week female Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Chow diet +/− 1% fish (n-3) or corn oil (n-6)</td>
<td>20</td>
<td>20</td>
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<td></td>
<td>8-week male Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>23% fat + 0.045% chol. +/− 1% fish (n-3) or corn oil (n-6)</td>
<td>20</td>
<td>20</td>
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<td>Yamashita et al. [16]</td>
<td>6-week male Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>40% lipid, 40% carbohydrates, 20% protein, 0.05% chol. + safflower oil (76% n-6 FA) + flaxseed oil (55% n-3 FA) (n-6:n-3 : 0.29, 1.43, 5 and 8)</td>
<td>16</td>
<td>16</td>
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<tr>
<td>Wang et al. [17]</td>
<td>10-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>Control vs Fish oil vs corn oil</td>
<td>10</td>
<td>10</td>
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<td>Adan et al. [18]</td>
<td>7-week male Apoe&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>9% olive oil, 43.75% sucrose, 1% chol. +/− 1% DHA or safflower</td>
<td>8</td>
<td>8</td>
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<td>Rudel et al. [19]</td>
<td>8-week male/female Ldlr&lt;sup&gt;−/−&lt;/sup&gt;</td>
<td>10% fat, 0.005% chol +/− saturated (palm oil), cis/trans mono, n-3 (fish oil), n-6 (safflower oil) polyunsaturated FA</td>
<td>8</td>
<td>8</td>
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<tr>
<td>Renier G. et al. [20]</td>
<td>6-week C57BL/6</td>
<td>Control vs /− 10% menhaden oil or 10% palm oil + 2% chol.</td>
<td>15</td>
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EPA : eicosapentaenoic acid, DHA : docosahexaenoic acid, ALA : α-linoleic acid (flaxseed oil, precursor of EPA, DPA and DHA), LA : linoleic acid, mΦ : macrophage : not described,
as inflammation in aortic lesions highlighting the major impact of n-3 FA on monocyte recruitment and subsequent macrophage accumulation in the arterial wall. However, although supplementation with n-3 FA allows an efficacious lowering of plasma lipid levels in humans [5], studies in mouse models suggest that the anti-atherogenic action of n-3 FA is independent of any effects on plasma cholesterol or triglyceride levels [7]. However, that must be asserted with caution as lipid metabolism is quite different in mouse in comparison to humans, highlighting the need to study in the future the effects of n-3 FA on atherosclerosis in a mouse model exhibiting a more “humanized” lipid metabolism as achieved in apoB/CETP mice.

In a previous issue of Atherosclerosis, Chang et al. [21] re-evaluate the impact of fish oil n-3 FA on atherosclerosis development by operating an incremental replacement of saturated fats (SAT) by n-3 FA (pure fish oil, EPA and DHA-rich) in LDLr−/− mice fed a high-fat (21%, w/w)/high-cholesterol (0.2%, w/w) diet for a 12-week period. This experimental approach is quite pertinent as dietary fat intake in developed countries, as in United States, derived mostly from saturated FA and is poor in n-3 FA. Then, using this strategy the authors were able to evaluate the potential beneficial effects of a supplementation with fish oil n-3 FA in a dietary context for which the FA intake is relevant.

Here, Chang et al. demonstrated that the progressive increase of dietary intake of fish oil n-3 FA (EPA and DHA) abrogated the deleterious effects of a SAT diet, thereby suggesting that a dietary n-3 FA intake on a SAT background is potentially efficacious. However that must be stated that the atheroprotective effects of n-3 FA need to be validated using humanized mouse models as in the model of apoB/CETP mice.

Analysis of LPL and macrophages in aortic roots from mice fed the different diets indicated that LPL expression colocalized with macrophages, an observation in agreement with the idea that LPL in atherosclerotic lesions is mostly expressed by macrophages [29]. Incremental replacement of SAT by fish oil n-3 FA strongly attenuated both macrophage and LPL contents in lesion areas. However the diminished amount of aortic LPL did not only result from the reduced number of macrophages as LPL mRNA levels were found decreased in aortic macrophages isolated by Laser Capture Microdissection (LCM). This observation clearly indicate that dietary n-3 FA intake not only hamper monocyte trafficking into the aorta [7], but also repress LPL expression by aortic macrophages. The inhibitory effect of n-3 FA on LPL expression by macrophages was previously observed [20] and was proposed to involve PPAR [30]. Because a decrease of PPARα expression paralleled that of LPL in arterial macrophages extracted by LCM, the authors speculate that PPARα might participate to the anti-atherogenic role of n-3 FA by regulation of LPL expression. Although such a mechanism still needs to be demonstrated, this hypothesis is not supported by a recent study indicating that activation of PPARα in macrophages reduced LPL activity through activation of Angiopoietin-like 4 (ANGPTL4) expression without having any effect on LPL mRNA levels [31]. In addition, coherent with the role of LPL in macrophage lipid uptake, activation of PPARα in this latter study inhibits foam formation. In some aspects, the reduction of aortic macrophage LPL expression by fish oil n-3 FA (EPA and DHA) observed by Chang et al. may appear surprising as EPA and DHA are natural ligands for PPARα [32] that is a potent activator of LPL expression [33]. Taken together with the observation that lipolysis of triglyceride-rich lipoproteins (TRL) by LPL generates free FA (FFA) acting as PPAR ligands able to trigger an anti-inflammatory response [34], that suggests that beneficial effects of the replacement of SAT by n-3 FA dependent on LPL likely do not require PPARs.

Among the various mechanisms by which n-3 FA exert anti-inflammatory properties [3], EPA and DHA repressed inflammation by shutting down NF-κB activation in macrophages. Since expression of TLR-4 and NF-κB target genes, IL-6 and TNFα, in aorta from mice fed diets containing n-3 FA were decreased when compared to SAT, those results strongly support the contention that n-3 FA repress inflammation by inhibiting the TLR4/NF-κB signaling cascade likely through the macrophage n-3 FA receptor GPR120 [35]. Although further studies are needed to explore the complete spectrum of actions of n-3 FA on atherosclerosis development and CVD, this study provides important information that suggests that n-3 FA intake is a pertinent strategy to reduce risk of CVD.

References

[8] Nakajima K, Yamashita T, Kita T, et al. Orally administered eicosapentaenoic acid induces rapid regression of atherosclerosis via modulating the phenotype of inflammation in aortic lesions highlighting the major impact of n-3 FA on monocyte recruitment and subsequent macrophage accumulation in the arterial wall. However, although supplementation with n-3 FA allows an efficacious lowering of plasma lipid levels in humans [5], studies in mouse models suggest that the anti-atherogenic action of n-3 FA is independent of any effects on plasma cholesterol or triglyceride levels [7]. However, that must be asserted with caution as lipid metabolism is quite different in mouse in comparison to humans, highlighting the need to study in the future the effects of n-3 FA on atherosclerosis in a mouse model exhibiting a more “humanized” lipid metabolism as achieved in apoB/CETP mice.

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