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Review

Long-chain omega 3 fatty acids: Molecular bases of potential antioxidant actions

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ABSTRACT

Several lines of investigation are being developed to assess the impact of polyunsaturated fatty acids, namely those of the omega 3 series, intake on oxidative stress. Keeping in mind that there might be a dose-response relation, in vivo and in vitro data strongly suggest that omega 3 fatty acids might act as anti- rather than pro-oxidant in several cells such as vascular cells, hence diminishing inflammation, oxidative stress, and, in turn, the risk of atherosclerosis and degenerative disorders such as cardiovascular disease.

1. Introduction

The oxidative hypothesis of atherosclerosis posits that lipid peroxidation contributes to the development of atherosclerotic plaques via formation of macrophage- and lipid-laden foam cells [1]. Even though the extent of oxidative stress' contribution to atheroma development is as yet to be fully ascertained, the formation of peroxidation products should, indeed, be limited to a minimum. In this respect, there is widespread concern that pharma-nutritional [2,3] intakes of polyunsaturated fatty acids (PUFAs, notably long chain omega 3 fatty acids such as eicosapentaenoic – EPA – and docosahexaenoic – DHA-acids) might increase circulating concentrations of lipoperoxides. Indeed, PUFAs are theoretically more susceptible to oxidation because oxygen easily attacks double bonds, yielding lipoperoxides [4]. These compounds, when generated in biological systems, are cytotoxic and, if not scavenged, may initiate a chain reaction (propagation). When it occurs in fats and oils, this process leads to rancidity. As a result, commercially-available supplements do contain marked amounts of lipoperoxides [5,6]. In humans, despite >20 years of extensive research, the biological relevance of oxidative damage in the onset and development of atherosclerotic lesions is not very clear [7]. As an example, the concomitant presence of peroxidized lipids and lipid-soluble antioxidants such as vitamin E in atherosclerotic plaques cannot be easily explained in biochemical terms [8]. In any case, even though we are still debating the extent and precise nature of this contribution, the fact that oxidative stress contributes to atherosclerosis development has been established [9].

Based on the considerations discussed above, several lines of investigation have been developed to assess the impact of PUFAs intake on oxidative stress. This paper reviews the available evidence and discusses the cellular pathways involved in the PUFAs-elicited antioxidant response.

1.1. In vivo

We will first discuss the human trials that tested the effects of omega 3 intake on systemic oxidative stress (Table 1). One important note of caution is that the methods we currently can rely on to measure oxidative stress in vivo are very limited. As reviewed by several authors, free radicals are extremely reactive molecules that are impossible to quantify in vivo [4,9]. Consequently, we can only evaluate the damage induced by free radicals and other oxidants to macromolecules, namely proteins and DNA, as well as lipids and sugars [10]. Moreover, dose-response studies of antioxidants yielded equivocal results (at best) [11], such that we still cannot precisely attribute clear antioxidant effects to individual molecules or raw mixtures. In other words, in the absence of robust biomarkers it becomes difficult to correctly evaluate the effects of drugs and nutrients. In addition, there is an unmet need to take into account the effects of polymorphisms [12], which might indeed affect the dose below which omega 3 fatty acids act as antioxidants and above which they could form lipoperoxides, potentially interfering with the immune system [13].

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Table 1
Human studies of long-chain omega 3 supplementation and oxidation markers.

Reference	Subjects	Treatment	Outcome
Higdon et al. [17]	Fifteen postmenopausal women, crossover trial	15 g of sunflower oil providing 12.3 g/day of oleate; safflower oil providing 10.5 g/day of linoleate; fish oil providing 2.0 g/day of EPA and 1.4 g/day of DHA. Five-week treatment.	Fish oil supplementation did not increase LDL oxidation in vivo, especially when compared with safflower supplementation.
Higdon et al. [17]	Fifteen postmenopausal women; crossover trial	15 g of sunflower oil providing 12.3 g/day of oleate; safflower oil providing 10.5 g/day of linoleate; fish oil providing 2.0 g/day of EPA and 1.4 g/day of DHA. Five-week treatment.	↓ plasma free F_2-isoprostane concentrations after fish-oil supplementation than after sunflower-oil supplementation.
Barden et al. [19]	Eighty-three pregnant atopic women	4 g daily of either fish oil ($n=40$) or olive oil ($n=43$) capsules, from 20 weeks gestation until delivery.	↓ plasma ($p<0.0001$) and urinary ($p=0.06$) F_2-isoprostanes after fish oil.
Mas et al. [21]	Two 6-week placebo-controlled interventions, Study A: overweight, dyslipidemic men; and Study B: treated-hypertensive Type 2 diabetic, patients.	4 g daily EPA and DHA, for six-weeks.	↓↓ plasma F_2-isoprostane by EPA (24% in Study A, 19% in Study B) and by DHA (14% in Study A, 23% in Study B)
Mori et al. [23]	55 untrained, sedentary, dyslipidemic NIDDM patients	Low-fat diet (30% of daily energy) with or without one daily fish meal (3.6 g omega-3 fatty acids per day) for eight weeks.	↓ (830 ± 321 pmol/24 h, or 20%) urinary F_2-isoprostane after fish relative to the low-fat diet alone
Guillot et al. [25]	Twelve healthy male volunteers (aged 53–65 yr)	200, 400, 800, and 1600 mg/d DHA for two weeks each dose.	↓ urinary isoprostanes after 200 mg/d but↑after 1600 mg/d DHA
Lee et al. [27]	67 mild cognitive impairment patients compared to those of 134 healthy elderly controls	Omega-3 PUFA intake assessed using an interviewer-administered food frequency questionnaire	↓ Plasma lipid hydroperoxide levels with increasing DHA and EPA intake

Nonetheless, one accepted biomarker of lipid peroxidation is the formation of isoprostanes, namely F_2-isoprostanes such as 8-iso-$PGF_{2\alpha}$ [14]. Currently, modulation of isoprostane formation and of their circulating concentrations and urinary excretion is the best marker of oxidant/antioxidant action. One important – though often overlooked – caveat is that F_2-isoprostanes are formed from arachidonic acid. Therefore, their circulating concentrations should be normalized to those of arachidonate [15], because omega 3 fatty acids might "just" displace 20:6ω6, thereby merely decreasing the substrate for F_2-isoprostanes formation.

The first reports of the effects of omega 3 fatty acids on systemic oxidative stress – evaluated as isoprostane excretion and LDL oxidizability, have been published by the Wander group in early '2000s [16,17]. Note that the authors also excluded a pro-oxidant effect of EPA and DHA on plasma proteins [18]. This issue has also been addressed by the Mori group, who also reported decreased isoprostane urinary excretion and plasma concentrations in fish- and fish oil-supplemented subjects [19–23]. Of note, this antioxidant effect was also observed in pregnancy [20]. Finally, the Lagarde and Calzada group proposed a threshold below which PUFA supplementation results in antioxidant actions and above which pro-oxidant activities take place [24–26]. Very recently, EPA and DHA supplementation was shown to lower plasma lipoperoxide concentrations in mild cognitive impairment patients [27]. Indeed, Yavin et al. proposed DHA as an antioxidant agent in the brain [28]. Very recently, Daak et al. [29] reported that DHA and EPA supplementation does not exacerbate oxidative stress or intravascular hemolysis in homozygous sickle cell patients, thus adding further evidence to the notion that omega 3 fatty acids are not pro-oxidant in humans.

Omega 3 PUFAs are also able to reduce lipoperoxidation levels, advanced glycation end products, SOD/CAT enzymatic ratio, and CAT immunocontent and increase SOD2 levels in the livers of diabetic rats fed with a high fat thermolyzed diet (rich in advanced glycation end-products) [30]. These properties, together with the reported inhibition of hepatic lipogenesis afforded by EPA and DHA [31], suggest a multifaceted healthful activity of omega 3 fatty acids supplementation in liver disorders.

1.2. *In vitro*

Based on mere chemical considerations, the susceptibility of fatty acids to oxidation is thought to be directly dependent on their degree of unsaturation with docosahexanoic acid being the most oxidizable one and palmitic acid being the least. However, under equal conditions of oxidative stress, fatty acids oxidize at a different rates and originate different oxidation products, in a manner that is unrelated to their degree of unsaturation, both from the qualitative and the quantitative points of view [32]. Of note, this behavior is seen when fatty acids are oxidized in an aqueous environment, which might or might not be relevant to human physiology (note, however, that lipoproteins and membrane fatty acids are located on lipid-water interfaces). It is also necessary to reiterate the need for more than one biomarker of lipid peroxidation, whose formation heavily depends on double bonds, oxidizing agent, etc. [4].

Another note of caution concerns the in vivo relevance of in vitro studies, notably because of the concentration issue. Some studies are being performed (Richard et al., in preparation), but results originating from cell culture studies should be interpreted with caution.

In a series of experiments, Richard et al. demonstrated that fatty acid micelles scavenge superoxide in an unsaturation-dependent manner, up to eicosapentaenoic acid, which is the most effective fatty acid [33]. Supplementation of human aortic endothelial cells (HAEC) with PUFAs of the omega 3 series leads to lower formation of ROS, as compared with cells supplemented with saturates, monounsaturates, or polyunsaturates of the omega 6 series. This effect is maximal at concentrations of 10 μM. The effects of omega 3 fatty acids on reactive species production appear to be stronger on ROS, as a milder, albeit significant effect is also observed on reactive nitrogen species (RNS) generation [33]. The endothelial cell data might partially explain the vascular antioxidant actions of fish oil reported by Casos et al. [34] and the amelioration of endothelial dysfunction in experimental menopause [35], when the risk of eNOS uncoupling increases, leading to higher ROS production [36,37]. DHA might prove useful in augmenting endothelial cells function [37] precisely because of its direct or indirect antioxidant activities.

To further investigate the molecular mechanisms responsible for the antioxidant effects, Richard et al. examined the actions of DHA on intracellular generation of ROS [38]. Among the cellular sources of oxidants, NADPH oxidases (Noxs) catalyze electron transfer from NADPH onto molecular O_2. This process generates reactive oxygen species (ROS), namely superoxide anion (O_2^-)