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n-3 polyunsaturated fatty acids - physiological relevance of dose

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Abstract

n-3 polyunsaturated fatty acids (PUFA) are widely used for chemotheraphy/chemoprevention of chronic diseases. However, the molecular mechanism(s) by which the bioactive n-3 PUFA (eicosapentaenoic acid and docosahexaenoic acid) modulate effector pathways are not fully elucidated. Multiple experimental approaches, including use of animal models, cell lines, and human clinical trials, have been utilized to dissect the complex effectors. It is imperative to link these different experimental approaches together in order to interpret outcomes in the context of human physiology and pathophysiology. Unfortunately, the adoption of a broad array of model systems and a wide range of fatty acid exposures (i.e. doses) has made it difficult to interpret biological outcomes. Therefore, in this mini-review we discuss the impact of (a) molecular structure of bioactive fatty acids, (b) dose relevance relative to human consumption, (c) enrichment of fatty acids in sera and tissues following dietary intake, and (d) limitations of cell/tissue culture studies.

Introduction

Long-chain polyunsaturated fatty acids (PUFA) are subcategorized into n-3 [alpha-linolenic acid (ALA, 18:3), eicosapentaenoic acid (EPA, 20:5), docosapentaenoic acid (DPA, 22:5), and docosahexaenoic acid (DHA, 22:6)] and n-6 [linoleic acid (LA, 18:2), arachidonic acid (ARA, 20:4)] families according to the position of the first double bond from the methyl end of the acyl chain. A plethora of data from epidemiological studies and clinical trials investigating the effect of increased consumption of n-3 PUFA either in the form of fish or fish oil supplements suggest that, compared to n-6 PUFA, n-3 PUFA favorably modulate multiple biological processes involved in coronary heart diseases [1,2], cancers [3–7], immune diseases [8–10], and brain health [11]. In general, studies involving cell culture and animal models utilizing fish oil, purified n-3 PUFA in triglyceride, free fatty acid or ethyl ester form, support the epidemiological and clinical observations [12–14]. However, the interpretation of experimental data with regard to physiological relevance is complicated by atleast two issues:

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(a) bioavailability/bioactivity of different forms of n-3 PUFA and (b) the dose and local concentration of effective n-3 PUFA in tissues. In this review, we will probe these issues specifically from the perspective of immune effector cell model systems.

Molecular forms of n-3 PUFA and their bioavailability

In processed fish oils available as supplements DHA is predominantly localized to the sn-2 position compared to EPA which is more randomly esterified to all three positions of the triglyceride backbone [15]. In this form, DHA is primarily absorbed as the monoglyceride [16]. This contrasts, for example, with seal oil (also rich in n-3 PUFA), where EPA, DPA and DHA are preferentially located both in the sn-1 and sn-3 positions in the triglyceride molecule [17]. Fatty acids can be easily released from the sn-1 and sn-3 positions by pancreatic lipase and are directly absorbed [15].

There are reports that fish consumption is more effective at increasing serum EPA and DHA levels in humans compared to supplementation with fish oil [18]. With respect to intramolecular fatty acid distribution, the randomization of n-3 PUFA within fish oil triglycerides does not appear to have an effect on the apparent digestibility of the individual fatty acids [17]. In addition, De Schrijver et al [19] concluded that once n-3 PUFA are absorbed, their effect on lipid metabolism in the rat is not determined by the dietary source. Manipulation of fatty acid content of an oil may increase the susceptibility of the oil to oxidation relative to its unmodified counterpart [20]. Interestingly, liposomes based on natural phospholipids enriched in n-3 PUFA may have enhanced bioavailability compared to standard fish oil [21]. Lastly, with respect to in vivo models to evaluate n-3 PUFA bioavailability, it is important to note that most of the studies conducted to date have been in rats.

What is a relevant dose of n-3 PUFA in experimental models?

In general, the typical American consumes 0.7–1.6 g of n-3 PUFA per day, equivalent to approximately 0.2–0.7% of total calories [22,23]. Much of this is as ALA, the plant n-3 PUFA, and intake of fish-derived long chain n-3 PUFA (i.e., EPA and DHA) was reported to be less than 0.1–0.2 g per day. In contrast, in human clinical trials, 1–9 g/day (0.45–4% of calories) n-3 PUFA, mainly in the form of EPA and/or DHA has been used [24–27]. With respect to physiological relevance, this range is similar to levels consumed by Greenland Inuit (i.e., 6–14 g/day, which corresponds to 2.7–6.3% of daily energy) [28,29]. Similarly, traditional Japanese diets contain 1–2% of daily energy as long chain n-3 PUFA [30,31]. Therefore, it seems reasonable for animal feeding studies designed to probe the biological properties of n-3 PUFA relevant to humans, that 4% (wt/wt) fish oil or 1% purified n-3 PUFA ethyl esters be used. This level of intake delivers ~2.4% of total energy as n-3 PUFA, which is within the range consumed by humans and used in human clinical trials.

Enrichment of DHA in serum and tissues

Conquer et al. reported the amount of n-3 PUFA in serum total phospholipids and non-esterified fatty acids (NEFA) in subjects supplemented with 1.5 g DHA/day (~0.7% of calories) [22]. The circulating phospholipid form of DHA (402 μM) was predominant in serum compared to NEFA (12.7 μM) (Table 1). Levels approximating 130 μM DHA in total phospholipids and 1.5 μM in NEFA were detected in the control group. Overall, DHA supplementation (at 1.5 g/day) increased phospholipid DHA 3-fold, compared to a 0.5-fold increase in EPA. In contrast to DHA enrichment in human sera, Switzer et al [13] demonstrated only a modest enrichment of DHA and DPA in mouse serum total phospholipids following consumption of a diet containing 4% (wt/wt) fish oil, which supplied 0.87% of total calories as DHA. Notably, EPA (9.9 μM) was significantly enriched in mouse serum compared to the n-6 PUFA rich corn oil fed control group (Table 1).

With regard to tissue enrichment following n-3 PUFA consumption, Damsgaard et al. demonstrated that DHA, DPA and EPA are highly enriched in human peripheral blood mononuclear cells (PBMC) following the intake of 5 mL fish oil/d for 8 wk (Table 2) [28]. Significant amounts of n-3 PUFA (DHA, DPA and EPA) were also observed in subjects consuming 5 mL olive oil (negative control), likely indicating the contribution of the previous dietary consumption of n-3 PUFA. In comparison, in the mouse model, we have shown that, following fish oil feeding (4%, wt/wt), $CD4^+$ T-cell membrane lipid raft and non-raft membrane fractions become enriched in DHA and EPA [13]. Fish oil supplementation increased mainly DHA levels in T-cell membrane rafts (4-fold) and non-rafts (1.9-fold) of $CD4^+$ T cells compared to T-cells from 5% corn oil fed mice. Leslie et al. [32] demonstrated that macrophages from mice fed a diet containing 5% fish oil were highly enriched in DHA (9.8 mol%), as well as in total n-3 PUFA (22.3 mol%), compared to 5% corn oil fed control mice (Table 2).

EPA/DHA enrichment in cell culture

Cell culture studies are convenient and advantageous in some circumstances. However, interpretation of cell culture data in terms of biological outcomes is not always straightforward, since the experimental conditions may be somewhat contrived and perhaps far removed with respect to physiological relevance. To assess the effect of dietary fatty acids on specific tissues, animals are typically fed n-3 PUFA enriched diets followed by the isolation of tissues/cells to be activated in media, ex vivo. However culture itself can modify the fatty acid composition of cells. Switzer et al. [13] demonstrated n-3 PUFA enrichment in the non-raft fraction of murine $CD4^+$ T-cells induced by 4% fish oil feeding for 2 wk dropped from 7.7 mol% to 4.1 mol% by culturing the cells in 5% fetal bovine serum supplemented culture medium for 5 d, whereas n-3 PUFA in the raft fraction remained at 2.2 mol% after culture. The loss of fatty acids in the culture might result in the loss of a diet-induced phenotype, and therefore, possible misinterpretation of the treatment effect. To overcome these limitations, cell culture in the presence of homologous serum in the medium has been used to maintain a significant amount of fatty acids in cell membranes [13]. Indeed, the n-3 PUFA level in the non-raft fraction of $CD4^+$ T-cells from 4% fish oil fed mice increased from 4.1 to 12.2 mol% following 5 d in culture with homologous serum. This also complicates interpretation, since it is difficult to rule out a direct effect of n-3 PUFA containing homologous serum. Of interest, recently, Fan et al. [33] demonstrated that $CD4^+$ T-cells from Fat-1 transgenic mice, which generate endogenous n-3 PUFA by n-3 desaturase, maintained the initial amount of n-3 PUFA in T-cell membranes after 72 h in culture without homologous serum supplementation. These data indicate that Fat-1-containing cells express a physiologically relevant, n-3 PUFA enriched, membrane fatty acid composition which is resistant to conventional cell culture-induced depletion.

Li et al. [34] reported enrichment of n-3 PUFA into both lipid raft and non-raft membrane phospholipid fractions upon incubation of human Jurkat $CD4^+$ T-cells with 50 μM DHA (Table 3). However, DHA content (15.3 mol% in rafts and 15.0 mol% in non-rafts) was higher compared to dietary enrichment of n-3 PUFA in human PBMC (10.0 mol% in total phospholipids) or murine $CD4^+$ T-cells (2.2 mol% in rafts and 7.7 mol% in non-rafts) as described above. Therefore, the effect of the concentration of fatty acids used in cell culture studies should be carefully considered with respect to physiological relevance. As a precautionary note involving n-3 PUFA enrichment in cells, we recently noted that “lipid bodies” form when human Jurkat $CD4^+$ T-cells are incubated in the presence of 50 μM DHA. This impairs the ability of these cells to form an immunological synapse with co-incubated human Raji B-cells (Figure 1). Therefore, investigators performing cell culture studies involving the supplementation of media with fatty acids should remain vigilant of the off-target and perhaps toxic effects of long chain PUFA in culture.

Conclusion

In this report, we reviewed the potential complicating effects of the molecular form and dose of n-3 PUFA on biological endpoints. Clearly, the interpretation of experimental outcomes can be confounded by the failure to consider the effects of the molecular form and the dose of the fatty acid used and also the incorporation into discrete intracellular domains.

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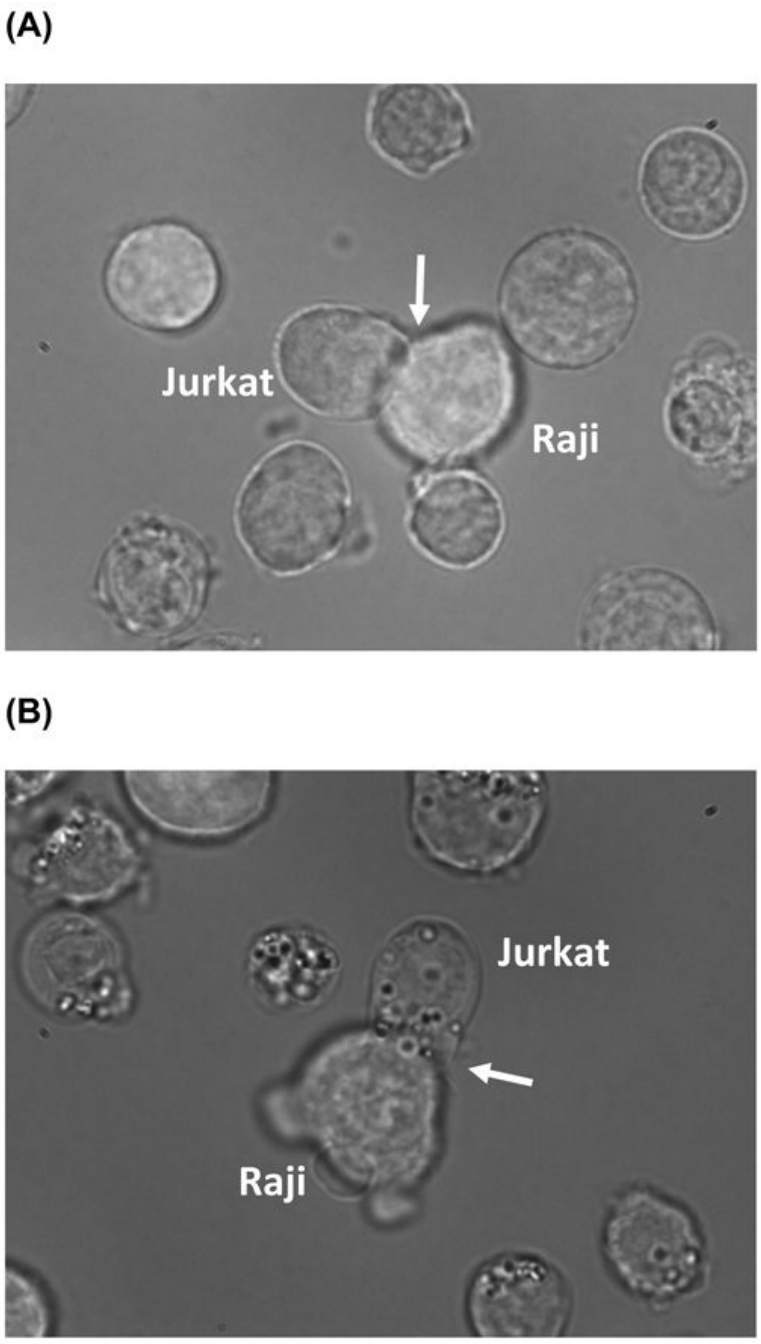

Figure 1.
The formation of "lipid bodies" following DHA incubation imaged by bright field microscopy. (A) Fetal bovine serum (FBS, control) or (B) 50 μM DHA was added to FBS (72 h) treated human Jurkat $CD4^+$ T-cells co-cultured with human Raji B-cells primed with superantigen Styphyllococal Enterotoxin E to form an immunological synapse (arrow).

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Table 1

Enrichment of n-3 PUFA in sera by dietary intake.

Model	Dose		Treatment	Control	References
Human serum	1.5 g DHA/d for 6 wk vs 0 g DHA	Serum total phospholipids	402 μM DHA	133 μM DHA	[22]
			7.9 mol% DHA	2.8 mol% DHA	
			0.5 mol% DPA	0.9 mol% DPA	
			1.5 mol% EPA	1.0 mol% EPA	
		Non-esterified fatty acid	12.7 μM DHA	1.5 μM DHA	
			2.2 mol% DHA	0. μmol% DHA	
			0.0 mol% DPA	0.0 mol% DPA	
			0.1 mol% EPA	0.1 mol% EPA	
Mouse serum	4% fish oil+ 1% corn oil for 2 wk vs 5% corn oil	Serum total phospholipids	6.7 μM DHA	6.7 μM DHA	[13]
			0.2 μM DPA	0.0 μM DPA	
			9.9 μM EPA	0.0 μM EPA	

Table 2

Enrichment of n-3 PUFA in immunocytes by dietary intake.

Model	Dose		Treatment	Control	References
Human PBMC	5 mL fish oil/d vs 5 mL olive oil	Total phospholipids	3.4 mol% DHA	2.7 mol% DHA	[28]
			4.0 mol% DPA	2.6 mol% DPA	
			2.6 mol% EPA	0.3 mol% EPA	
			10.0 mol% n-3 PUFA	5.6 mol % n-3 PUFA	
Mouse $CD4^+$ T-cells	4% fish oil + 1% corn oil for 2 wk vs 5% corn oil	Phospholipid in raft fraction	2.5 mol% DHA	0.6 mol% DHA	[13]
			0.8 mol% EPA	0.0 mol% EPA	
		Phospholipid in non-raft fraction	4.5 mol% DHA	1.3 mol% DHA	
Mouse macrophages	5% fish oil for 9 wk vs 5% corn oil	Total phospholipids in macrophages	9.8 mol% DHA	0.1 mol% DHA	[32]
			22.3 mol% n-3 PUFA	0.1 mol% n-3 PUFA	

Table 3

n-3 PUFA enrichment in human Jurkat CD4$^+$ T-cells by DHA treatment in cell culture.

Dose		Treatment	Control	References
50 μM DHA for 48 h vs 50 μM AA	Phospholipid in raft fraction	15.3 mol% DHA	0.3 mol% DHA	[34]
		1.0 mol% DPA	0.3 mol% DPA	
		1.2 mol% EPA	0.6 mol% EPA	
	Phospholipid in non-raft fraction	15.0 mol% DHA	3.0 mol% DHA	
		0.0 mol% DPA	0.7 mol% DPA	
		0.0 mol% EPA	0.0 mol% EPA	

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