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Review Article

Bioactive dietary long-chain fatty acids: emerging mechanisms of action

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The plasma membranes of all eukaryotic cells contain heterogeneous self-organising intrinsically unstable liquid ordered domains or lipid assemblies in which key signal transduction proteins are localised. These assemblies are classified as 'lipid rafts' (10–200 nm), which are composed mostly of cholesterol and sphingolipid microdomains and therefore do not integrate well into the fluid phospholipid bilayers. In addition, caveolae represent a subtype of lipid raft macrodomain that form flask-shaped membrane invaginations containing structural proteins, i.e. caveolins. With respect to the diverse biological effects of long-chain PUFA, increasing evidence suggests that *n*-3 PUFA and perhaps conjugated fatty acids uniquely alter the basic properties of cell membranes. Because of its polyunsaturation, DHA and possibly conjugated linoleic acid are sterically incompatible with sphingolipid and cholesterol and, therefore, appear to alter lipid raft behaviour and protein function. The present review examines the evidence indicating that dietary sources of *n*-3 PUFA can profoundly alter the biochemical make up of lipid rafts/caveolae microdomains, thereby influencing cell signalling, protein trafficking and cell cytokinetics.

Membrane rafts: *n*-3 Fatty acids: Conjugated fatty acids: Microdomains

The balance between cell proliferation and apoptosis is critical to the maintenance of steady-state cell populations in the body. In general, dysregulation of this mechanism can disrupt homeostasis, resulting in clonal expansion, with the resultant overproduction of affected cells. The programmed induction of cell death also represents a mechanism by which inappropriately activated cells and cells possessing DNA damage can be deleted. It has now been clearly established that chronic inflammation can perturb cellular homeostasis and drive malignant transformation by progressively inhibiting apoptosis of target cell types, for example, T-cells and epithelial cells[1,2]. Hence, chemotherapeutic agents such as dietary long-chain *n*-3 PUFA and possibly conjugated fatty acid species, which restore the normal proliferative and apoptotic pathways, have the potential for effectively treating cancers that depend on aberrations of these pathways to stay alive[3]. The following sections describe a mechanistic membrane-based model which may in part explain the pleiotropic properties of bioactive PUFA.

n-3 Polyunsaturated fatty acids

Although beyond the scope of the present review, a number of investigators have recently addressed the role of *n*-3 PUFA in suppressing chronic inflammation and cancer[4–6]. With respect to mechanisms which functionally link the pleiotropic effects of bioactive dietary long-chain *n*-3 PUFA, inflammation and cancer, examples include (i) metabolic interconversion into novel bioactive eicosanoids[7,8], (ii) modulation of nuclear receptor activation, gene transcription and translation[9–12] (Fig. 1), (iii) alteration of membrane phospholipid composition and functionality of self-organising lipid domains[13] (Fig. 2), (iv) effects on protein trafficking, including cytosol-to-membrane translocation[4,14] and (v) interaction with SCFA to trigger lipid oxidation and intracellular Ca^{2+} compartmentalisation[15,16].

The health benefits of long-chain PUFA are diverse and nutritional studies continue to demonstrate important benefits from the consumption of *n*-3 PUFA[4,7,8,17–19]. Since the use of a health claim on labels for foods containing *n*-3 PUFA has been approved, food companies are now mobilising to incorporate these fatty acids into a range of novel commercial foods in order to provide for the wider public consumption of these bioactive compounds. Hence, it is now important to precisely determine how specific long-chain PUFA modulate cell phenotype and reduce the risk of developing cancer and inflammatory disorders.

Abbreviation: CLA, conjugated linoleic acid.

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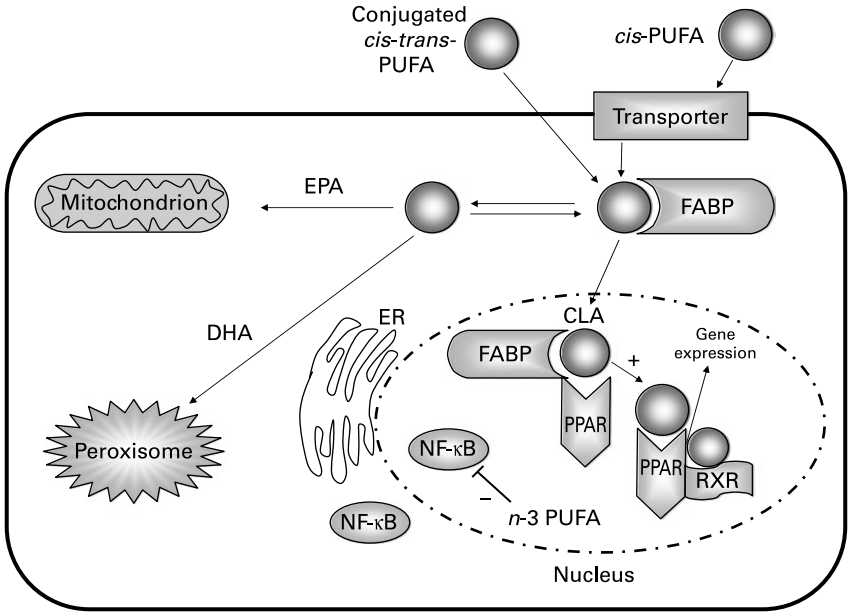

Fig. 1. Nuclear receptor activation by conjugated linoleic acid (CLA). FABP, fatty acid-binding proteins (molecular chaperone); ER, endoplasmic reticulum; RXR, retinoid X receptors. CLA transactivates PPAR nuclear receptors. *n*-3 PUFA suppress NF-κB activation. All membranes incorporate EPA, DHA and conjugated PUFA to different degrees.

Conjugated linoleic acid

There is growing interest with regard to the use and commercial availability of conjugated positional and geometric isomers of PUFA, particularly conjugated dienoic isomers of linoleic acid (conjugated linoleic acid; CLA). For example, in certain model systems, CLA is a powerful anti-cancer agent, capable of promoting growth arrest and apoptosis in tumour cells[20–22]. In addition, CLA triggers adipose delipidation in rodent species[23–25], and although there has been very little published clinical research[26,27], recent preliminary evidence suggests that mixed-isomer CLA supplementation can alter fat oxidation and energy expenditure in human subjects[28,29]. However, the precise mechanism of action

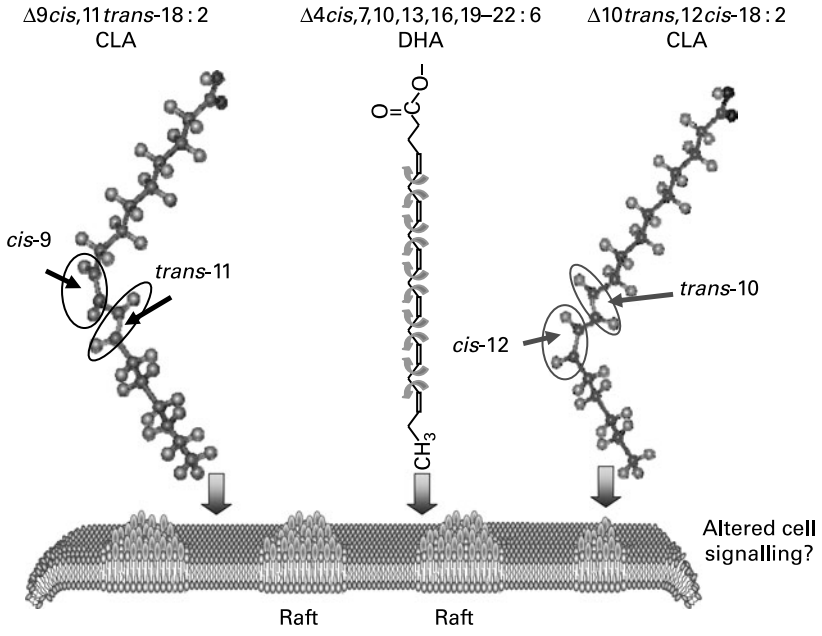

Fig. 2. Putative membrane microdomain-altering properties of *n*-3 PUFA and conjugated linoleic acid (CLA). Dietary DHA and CLA are incorporated into both the bulk phase of the plasma membrane as well as discrete heterogeneous cholesterol/sphingolipid-rich raft domains. This can alter plasma membrane organisation of inner leaflets and the dynamic partitioning of transduction proteins, thereby modulating their function.

remains elusive. Since *n*-3 PUFA and CLA exhibit overlapping phenotypic properties, we propose a unifying molecular mechanism which may in part explain their protective effects.

Effect of bioactive polyunsaturated fatty acids on cell membranes

It is generally believed that the plasma membrane consists of a mosaic of functional microdomains that facilitate interactions between resident proteins and lipids[30,31]. Visible examples of these include caveolae, flask-shaped invaginations containing the structural protein caveolin-1 and many signal transduction proteins[32]. In addition, morphologically heterogeneous featureless microdomains, consisting mostly of cholesterol and sphingolipids, unable to integrate well into the fluid phospholipid bilayers, exist as 'lipid rafts'[33]. Although the existence of lipid rafts is still debated, new sophisticated imaging approaches have started to define cell surface nanoscale organisation[31]. Significantly, both cholesterol-dependent microdomains, analogous to lipid rafts, and non-raft signalling microdomains have been observed using electron microscopic imaging of two-dimensional plasma membrane sheets[34]. These studies have provided a template for further investigation into the effects of dietary PUFA on cell surface organisation and cell cytokinetics, apoptosis and disease progression.

With respect to the biological effects of *n*-3 PUFA, increasing evidence suggests that DHA is a unique fatty acid because it significantly alters basic properties of cell membranes, including acyl (ester-linked fatty acid) chain order and fluidity, phase behaviour, elastic compressibility, ion permeability, fusion, rapid flip-flop and resident protein function[35]. In part, due to the number of *cis* double bonds, DHA is sterically incompatible with sphingolipid and cholesterol and, therefore, appears to alter lipid raft behaviour[35]. Interestingly, a number of studies have recently demonstrated that dietary *n*-3 PUFA are incorporated into diverse cell types and appear to uniquely modulate cell membrane microdomains[13,36–39]. Indeed, we recently demonstrated that *n*-3 PUFA feeding can markedly alter lipid/protein composition of mouse colonic caveolae microdomains, thereby selectively modulating the localisation and function of caveolar proteins[13,14,37]. In addition, we demonstrated that H-Ras and endothelial NO synthase are displaced from caveolae in *n*-3 PUFA-fed mice, which was associated with the suppression of Ras-dependent signalling. In contrast, localisation of non-caveolae-resident proteins, K-ras and clathrin, was not affected, indicating selective displacement of acylated signalling proteins from caveolae by *n*-3 PUFA. Our findings highlight a novel modality by which *n*-3 PUFA influence membrane micro-organisation, thereby modulating biological responses.

Using T-cell-culture models, Stulnig *et al.* were the first to document the ability of PUFA enrichment to selectively modify the cytoplasmic layer of lipid rafts[40,41]. In complementary experiments, we investigated the effect of dietary *n*-3 PUFA on cholesterol/sphingolipid-rich plasma membrane microdomains (i.e. rafts) in mouse splenic T-cells[5,42,43]. A very novel and unexpected outcome from this effort was the demonstration that dietary *n*-3 PUFA reduced (by about 45 %) lipid raft sphingolipid content and altered raft fatty acid composition[36,44,45]. Therefore, we hypothesised that PUFA classes (*n*-6 *v.* *n*-3) differentially modulate T-cell membrane microdomains, which is supported by recent studies indicating that stimulation-induced protein kinase C θ translocation into T-cell lipid rafts is suppressed by dietary *n*-3 PUFA[36]. In addition, in an attempt to further probe the effects of DHA on protein kinase C θ effector pathway signalling, we have recently demonstrated that the diet modification of lipid rafts is associated with the suppression of transcription factor NF-κB, AP-1 transcription site activation, IL-2 secretion and lymphoproliferation[36]. With respect to lymphocyte subsets, recent studies indicate that the macromolecular complex organisation in lipid rafts is distinct in non-polarised, T helper cell, Th1 and Th2 polarised subsets[46]. This would suggest that these subsets, i.e. regulators of cell-mediated immunity (Th1) and humoral immunity (Th2), would respond differently to dietary PUFA-induced perturbation. However, the ability of DHA to influence membrane raft-mediated signalling in polarised T-cells has not been determined to date.

There is cogent evidence indicating that lipid raft integrity is a prerequisite for optimised signalling between T-cells and antigen-presenting cells[47,48]. In addition, recent studies suggest that long-chain PUFA can block antigen presentation by interfering with lipid raft-dependent formation of the immunological synapse[38,39,49]. Overall, these findings provide evidence indicating that dietary *n*-3 PUFA can profoundly alter the biochemical make up of cell membrane lipid rafts/caveolae microdomains, which may directly or indirectly influence membrane fusion and cell–cell signalling. Interestingly, only a single study to date has examined the effects of CLA with regard to lipid raft/caveolae composition. Huot & Ma[50] demonstrated that mixed CLA isomers are concentrated in caveolae phospholipids, resulting in the reduction of caveolae-resident proteins, caveolin-1 and Her-2/neu, in Michigan Cancer Foundation (MCF)-7 breast cancer cells. Unfortunately, few studies to date have assessed the physical properties of CLA isomers[51]. Therefore, future studies using purified CLA isomers are needed in order to elucidate how conjugated fatty acid structure affects membrane structure and function.

Docosahexaenoic acid alters the size and distribution of lipid rafts

In a proof of principle study, we sought to determine the effect of DHA on the size and distribution of lipid rafts *in vivo* [17]. Using immunogold electron microscopy of plasma membrane sheets coupled with spatial point analysis, morphologically featureless microdomains were visualised in HeLa cells. Cells were transfected with green fluorescent protein truncated H-ras (GFP-tH), which is located exclusively to inner leaflet rafts, and subsequently incubated with DHA and control fatty acid, for example, oleic acid (18 : 1*n*-9) for 48 h. Univariate K-function analysis of GFP-tH (5 nm gold) revealed that the interparticle distance was significantly reduced by DHA treatment compared with control fatty acid, indicating that select PUFA can increase clustering of proteins in cholesterol-dependent microdomains (GFP-tH), whereas non-raft microdomains are insensitive to DHA modulation. These novel findings suggest that the plasma membrane organisation of inner leaflets is fundamentally altered by DHA enrichment (Fig. 2).

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'Cholesterol-centric' view of membranes

SFA compared with PUFA have a preferential affinity for cholesterol. This relationship provides the basis for a lipid-driven mechanism for the lateral segregation of membrane elements into cholesterol-rich and -poor microdomains[35,52–55]. For example, unfavourable interaction between cholesterol and PUFA chains has been clearly demonstrated by the exclusion of cholesterol from dipolyunsaturated phosphatidylcholine membranes where it is forced to directly contact polyunsaturated chains. Studies using a variety of techniques including differential scanning calorimetry[56], ^{1}H NMR and nuclear Overhauser enhancement spectroscopy with magic angle spinning[52], determination of partition coefficients[57], measurements of lateral compressibility[58] and fluorescence anisotropy[53,59] indicate that the poor affinity of DHA and perhaps CLA for cholesterol provides a lipid-driven mechanism for lateral phase separation of cholesterol-rich lipid microdomains from the surrounding bulk membrane. This could in principle alter the size, stability and distribution of cell surface lipid microdomains such as rafts. Indeed, growing evidence from model membrane studies suggest that the energetically less favourable interaction between cholesterol and PUFA, especially DHA, promotes lateral phase segregation into sterol-poor/PUFA-rich and sterol-rich/SFA-rich microdomains[52,53,57,60–62].

Huang & Feigenson proposed the umbrella model to describe the solubility and condensing effect of cholesterol within membranes[63]. According to this model, phospholipid head groups act as 'umbrellas' to prevent the energetically unfavourable contact of the non-polar part of cholesterol with interfacial water. This shielding will be less effective for DHA-containing phospholipids with a large molecular cross-sectional area, facilitating cholesterol precipitation at a lower concentration. In addition, this model allows for speculation that phosphatidylethanolamine with a smaller head group may enhance the DHA-associated reduction in shielding effects relative to phosphatidylcholine. Consistent with this notion, unlike phosphatidylcholine bilayers where a marked reduction in cholesterol solubility requires polyunsaturation at both *sn*-1 and *sn*-2 positions, DHA at the *sn*-2 position with a saturated *sn*-1 chain is sufficient in phosphatidylethanolamine to trigger cholesterol precipitation[64].

Conjugated *n*-3 polyunsaturated fatty acids

There is growing evidence that the combination of conjugated double bonds and *n*-3 PUFA may have enhanced chemoprotective properties. Recent studies using a number of model systems suggest that conjugated EPA and conjugated DHA suppress tumour growth[65,66], suppress topoisomerases[67], induce apoptosis[68,69], inhibit lipid accumulation[70] and have potential use as therapeutic dietary supplements for minimising tumour angiogenesis[68,71]. Typically, conjugated EPA and conjugated DHA are generated by alkaline isomerisation, producing a mixture of isomers with conjugated double bonds, although small amounts are found in marine algae and seal oil. Since the exact structure of these novel fatty acid species has not been fully characterised, future studies are required to verify their safety and efficacy in humans. In addition, it remains to be determined whether conjugated EPA and conjugated DHA alter the size and distribution of cell surface microdomains.

Conclusion

A growing body of literature supports the contention that bioactive food components containing *n*-3 PUFA are important in suppressing chronic inflammation and cancer. Although the mechanism of EPA and DHA action is still not fully defined in molecular terms, it is becoming increasingly clear that *n*-3 PUFA alter cell membrane lipid microdomain composition, thereby favourably modulating the relay of extracellular signals from surface receptors to downstream signalling networks. Clearly, further studies are needed to clarify the nature of lipid rafts and the biological role of conjugated fatty acid species, including CLA, conjugated EPA and conjugated DHA families.

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