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$n-3$ PUFA and membrane microdomains: a new frontier in bioactive lipid research

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Abstract

In recent years, our understanding of the plasma membrane has changed considerably as our knowledge of lipid microdomains has expanded. Lipid microdomains include structures known as lipid rafts and caveolae, which are readily identified by their unique lipid constituents. Cholesterol, sphingolipids and phospholipids with saturated fatty acyl chain moieties are highly enriched in these lipid microdomains. Lipid rafts and caveolae have been shown to play an important role in the compartmentalization, modulation and integration of cell signaling. Therefore, these microdomains may have an influential role in human disease. Dietary $n-3$ polyunsaturated fatty acids (PUFA) ameliorate a number of human diseases including coronary heart disease, autoimmune and inflammatory disorders, diabetes, obesity and cancer, which has been generally linked to its membrane remodeling properties. Recent in vitro evidence suggests that perturbations in membrane composition alter the function of resident proteins and, consequently, cellular responses. This review examines the role of $n-3$ PUFA in modulating the lipid composition and functionality of lipid microdomains and its potential significance to human health.

Keywords: Caveolae; Caveolin-1; Cholesterol; Colon; EPA; DHA; Lipid rafts; $n-3$ PUFA; T cells

1. Introduction

Dietary fat plays an important role in human health and disease. Fat is a source of energy, but it is also a vital structural component of cellular membranes and is involved in many important cell-signaling pathways. The latter two points are the focus of this review, which examines the role of dietary fats such as $n-3$ polyunsaturated fatty acids (PUFA) in modulating plasma membrane composition and cellular signaling in relation to lipid microdomains. Dietary fat is perceived to contribute negatively to many diseases such as heart disease, diabetes, obesity and cancer. In contrast, there is a growing body of evidence indicating a protective effect of $n-3$ PUFA; however, a comprehensive understanding of the mechanism(s) of action has yet to be elucidated [1–3]. The primary source of very long chain $n-3$ PUFA in the diet is derived from the consumption of cold-water fatty fish such as salmon and tuna. Fish are rich in two specific $n-3$ PUFA, eicosapentaenoic acid (EPA; 20:5 $n-3$) and docosahexaenoic acid (DHA; 22:6 $n-3$). The broad health benefits of $n-3$ PUFA in many diseases, that is, heart disease, diabetes, obesity, cancer and autoimmune and inflammatory disorders, suggest that a common fundamental mechanism is involved. We suggest that lipid microdomains may be a novel modality mediating the biological effects of $n-3$ PUFA across a number of diverse organ systems.

2. Lipid rafts, caveolae and biological relevance

The once simple fluid mosaic model of the plasma membrane has evolved significantly and is now known to contain specialized microdomains such as lipid rafts and caveolae (Fig. 1). Other common names include detergent-

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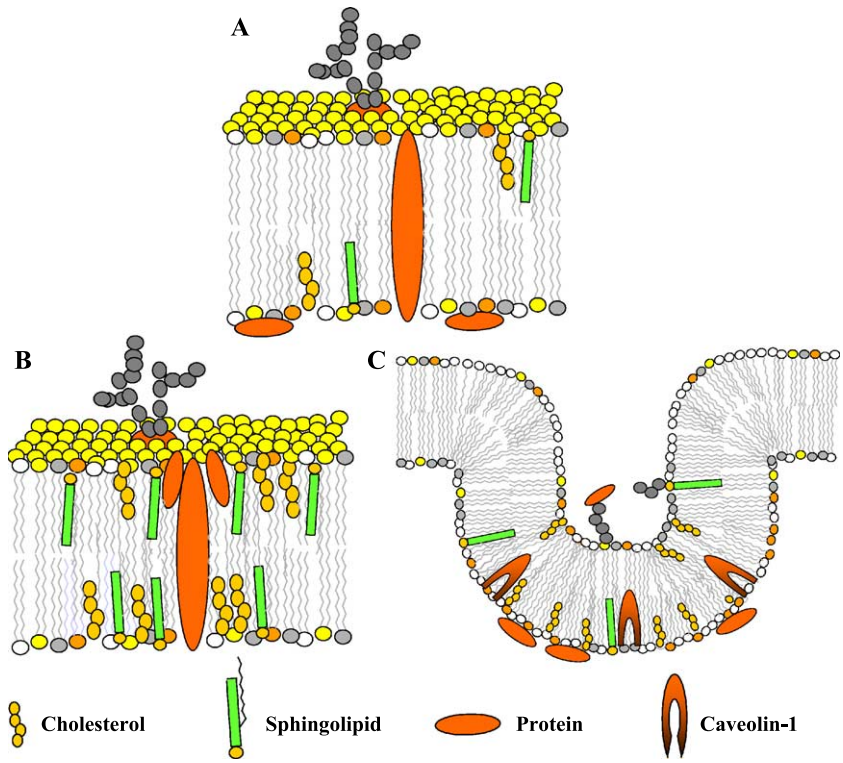

Fig. 1. Schematic representation of (A) a fluid mosaic model, that is, plasma membrane containing phospholipids, cholesterol, sphingolipids and protein in dynamic equilibrium. (B) Lipid raft enriched with cholesterol and sphingolipids. (C) Caveola enriched with caveolin-1, cholesterol and sphingolipid.

resistant membranes (DRMs) and detergent-insoluble glycosphingolipids. These descriptors reflect the insolubility of lipid domains in cold nonionic detergents. Our understanding of these microdomains has grown tremendously in the last several years [4]. The biochemical characterization of lipid rafts and caveolae has provided new insight into the regulation and function of plasma membrane proteins, which are regulated by differential segregation between the bulk membrane and specialized lipid microdomains.

Lipid rafts are a well-studied microdomain characterized by a "liquid-ordered" phase structure [5]. Lipid rafts consist of cholesterol tightly packed with sphingolipids, which do not integrate well into the fluid phospholipid bilayer, causing them to form microdomains [6,7]. However, there is still considerable controversy surrounding the basic physical properties of these domains, that is, compositional diversity, structure, size and dynamics. In contrast, the bulk membrane exists in a liquid crystalline, loosely disordered state due to the presence of unsaturated, kinked fatty acyl chains. The unique lipid raft environment attracts key signaling proteins [6,7]. These resident proteins include glycosylphosphatidylinositol-anchored proteins [8]; acylated proteins such as Src family kinases, heterotrimeric G proteins and endothelial nitric oxide synthase (eNOS) [9–11]; and palmitoylated transmembrane proteins such as β-secretase [12].

Raft function has been studied extensively in immune cells. Under basal conditions, individual components of the T cell receptor (TcR) and accessory molecules reside in small discrete rafts. Upon stimulation and activation of T cells, individual rafts coalesce to bring the activated TcR and associated signal-transducing molecules into proximity, thus providing an environment conducive to signal transduction [13]. For example, early mediators of T-cell proliferation such as protein kinase C theta (PKCθ), phospholipase C-gamma and linker for activation of T cells (LAT) and mediators of T cell apoptosis such as Fas and Fas ligand translocate to lipid rafts after stimulation [14–16]. In addition, an essential negative regulator of T-cell activation, cytotoxic T-lymphocyte-associated antigen 4, is recruited to the raft and down-regulates T-cell activation. [17,18]. These data describe an emerging paradigm that lipid rafts cluster at the T-cell/antigen-presenting cell interface, ultimately generating platforms specialized for sustained TcR signaling [19]. Overall, there is strong evidence that lipid raft integrity is required for optimal TcR signal transduction and immune response [19–22].

Caveolae are specialized rafts enriched with the structural protein caveolin-1 [23,24]. Caveolae are recognized in electron micrographs as flask-shaped structures that have a striated coat composed of caveolin-1 [25]. Caveolae have several functions including endocytosis, intracellular and extracellular cholesterol transport and signal transduction [23]. In particular, caveolae, due to their unique lipid environment, function as signaling platforms by concentrating lipid-modified proteins with saturated fatty acyl anchors. For example, the biochemical isolation of caveolae revealed the colocalization of many cell-signaling proteins including

eNOS and H-Ras [26]. This interaction may also be mediated by direct binding of signaling proteins to caveolin-1. Caveolae formation and function are dependent on both caveolin-1 and cholesterol. Depletion of membrane cholesterol by chemical agents such as cyclodextrin, filipin or nystatin inhibits caveolae formation [27,28]. Likewise, caveolin-1 knockout mice lack caveolae [26], and the expression of caveolin-1 in cells lacking caveolae induces caveolar formation [29].

It is clear that rafts are important regulators of cellular activity. New proteins associated with membrane microdomains are continually being identified. Several groups have utilized proteomics to identify several hundreds of proteins in lipid microdomains. Foster et al. [30] were one of the first groups to report the identification of 241 raft-specific proteins by proteomics. Similarly, Blonder et al. [31] recently reported the identification of 380 unique proteins from DRM rafts isolated from Vero (monkey kidney cells). Microdomain localization of many signaling proteins implicated in numerous human diseases suggests that lipid microdomains may play a potential role in chronic disease development and treatment (Table 1). With regard to nutritional modulation, it is well accepted that cellular membrane lipids can be remodeled by altering dietary fat intake, which consequently affects the physical property of membranes and membrane resident protein functionality [32,33]. Recent data have shown that select classes of fatty acids can also remodel microdomains. Described below are a number of studies that highlight the potential of $n-3$ PUFA to modulate lipid microdomain function in both in vitro and in vivo models.

3. In vivo effect of $n-3$ PUFA on T-cell lipid rafts

Early in vitro observations suggest that lipid rafts are sensitive to bilayer remodeling by manipulating the type of PUFA provided to cells in culture. PUFA incorporation into membrane lipids in a Jurkat T-cell line selectively modified lipid rafts and suppressed signal transduction by displacement of Src family kinases Lck and Fyn [34,35] and LAT [36] from lipid rafts. Similarly, in COS-1 cells, the localization of Fyn was shown to be inhibited by arachidonic acid (AA), EPA and DHA [37]. In general, these studies have shown that microdomain lipid composition and raft protein function can be altered by $n-3$ PUFA in vitro; however, the biological relevance of these observations requires validation from in vivo data. Recently, Fan et al. [38] provided substantive in vivo evidence in a mouse model demonstrating that raft fatty acyl lipid composition and sphingolipid content were markedly altered by an $n-3$ PUFA diet containing EPA and DHA, relative to an $n-6$ PUFA diet enriched with linoleic acid (18:2 $n-6$). Furthermore, changes in raft lipid composition were associated with a decrease in PKCθ translocation into T-cell lipid rafts by dietary $n-3$ PUFA relative to $n-6$ PUFA-fed mice [64]. Therefore, based on in vivo observations, which are largely consistent with the in vitro data, it is apparent that dietary sources of $n-3$ PUFA have a relevant biological role in modulating T-cell function via rafts.

In addition to T-cell signaling, there is also in vivo experimental evidence demonstrating that T-cell apoptosis, that is, activation-induced cell death (AICD), which is mediated by raft structure, is indirectly influenced by $n-3$ PUFA [39]. It has recently been reported that interferon (IFN)-γ is required for T cell AICD [40]. It is also known that the IFN-γ receptor (IFN-γR) is recruited to raft-like domains in T cells following IFN-γ stimulation [41]. Since dietary $n-3$ PUFA clearly remodel T-cell lipid rafts, one can speculate that $n-3$ PUFA may modulate IFN-γ signaling by enhancing IFN-γR raft localization due to alterations in raft composition. Clearly, additional studies are needed to expand upon these observations.

Table 1
Diseases and cellular targets potentially modulated by rafts and caveolae

Alzheimer's disease and Parkinson's disease [12,51,52]
Diabetes and obesity [53–56]
Cancer [57,58]
Atherosclerosis [59]
Immune cells [38,39,60]
Infections by virus, bacteria and pathogen [61–63]

4. $n-3$ PUFA alter intestinal caveolae lipid composition and resident protein localization in vivo

In an in vivo model, Ma et al. [42] have recently shown that dietary $n-3$ PUFA markedly alter the lipid composition of colonic caveolae/lipid rafts in mice fed with fish oil enriched with EPA and DHA, relative to mice fed with an $n-6$ PUFA (control) diet primarily containing linoleic acid (18:2 $n-6$). The $n-3$ fatty acids, EPA and DHA, from fish oil were incorporated into the fatty acyl groups of caveolae phospholipids and decreased the caveolar content of cholesterol and caveolin-1, the major structural component of caveolae by ~50%. The effect of diet on caveolar cholesterol content is particularly notable since $n-3$ PUFA, unlike cholesterol-sequestering reagents that have been used to perturb microdomain integrity in vitro, selectively reduced caveolar cholesterol content without affecting total cellular cholesterol. Pharmacological depletion of total cellular cholesterol content not only perturbs cholesterol-rich microdomains but also nonspecifically alters many other cellular functions requiring cholesterol. Concomitantly, alterations in the caveolar microenvironment by $n-3$ PUFA selectively inhibited the localization of caveolae-targeted proteins, H-Ras and eNOS, to caveolae, whereas the localization of non-caveolae proteins, K-Ras and clathrin, was unchanged [42]. Moreover, epidermal growth factor-induced activation of H-Ras, but not of K-Ras, was significantly decreased following $n-3$ PUFA treatment, in parallel with differential alterations in their microlocalization. These observations are

consistent with in vitro studies demonstrating that H-Ras, but not K-Ras, requires proper access to lipid rafts and caveolae for its full activation [43,44].

The above-mentioned in vivo study has important biological implications. It highlights the potential impact of modulating dietary lipid composition as a means to selectively alter protein function in vivo. Considering the growing list of signaling proteins residing in lipid microdomains, we anticipate that further studies on the effects of $n-3$ PUFA on lipid microdomains may help develop a new paradigm for understanding the complexity of $n-3$ PUFA modulation of signaling networks.

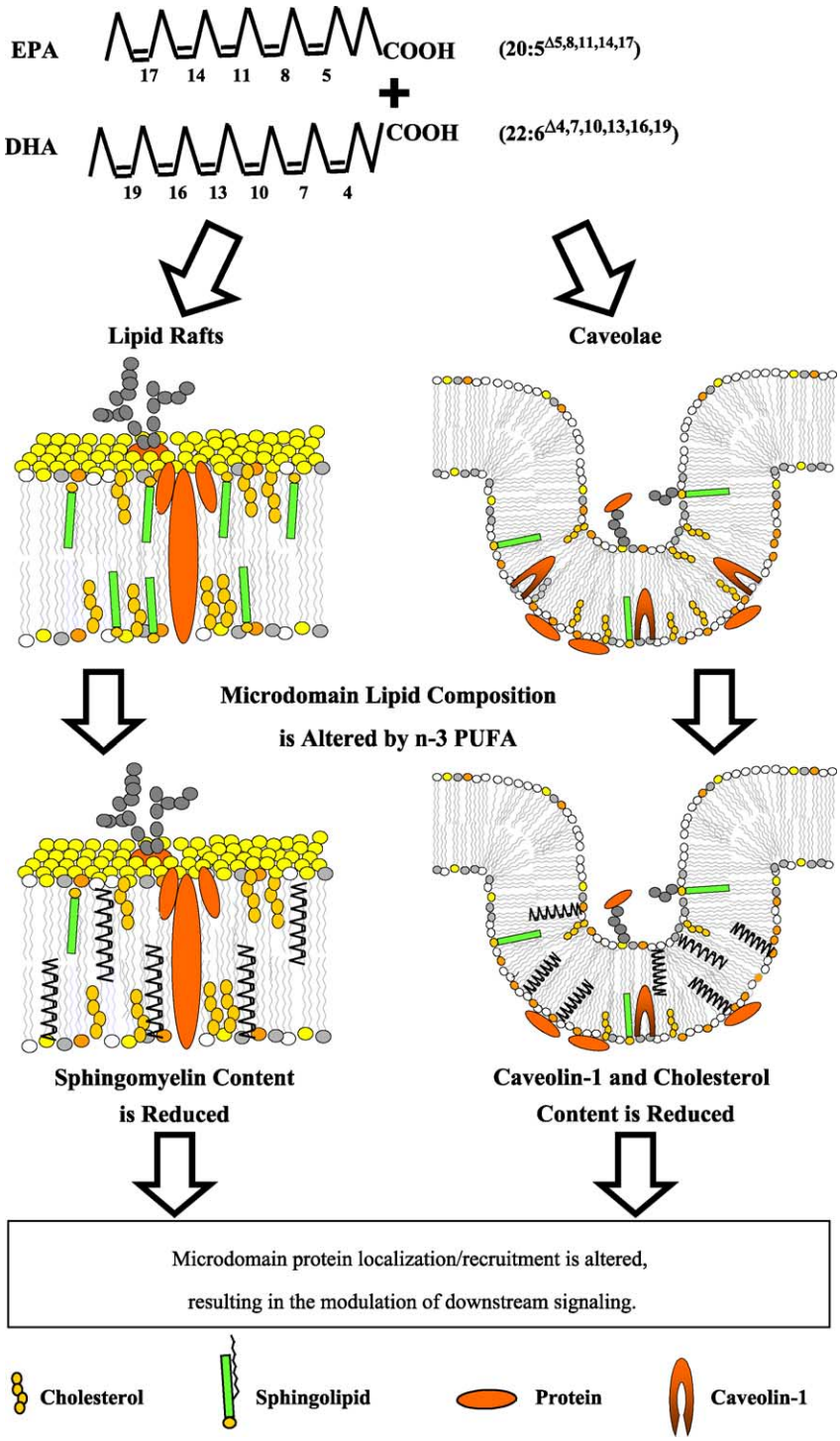

Fig. 2. Dietary $n-3$ PUFA readily incorporate into lipid rafts and caveolae. EPA and DHA are acylated to phospholipids within both rafts and caveolae. The presence of highly flexible, very long chain unsaturated fatty acids is not compatible with the ordered phase properties of rafts and caveolae. Consequently, the localization of microdomain-specific proteins is perturbed, resulting in the modulation of downstream cellular signaling events.

5. Mechanism of *n*−3 PUFA action

n−3 PUFA incorporation into lipid microdomains has been shown to coincide with reductions in cholesterol or sphingomyelin content therein, which are important in the assembly of a liquid-ordered phase structure. *n*−3 PUFA-induced caveolar cholesterol reduction may be explained, at least in part, by poor solubility of cholesterol in phospholipids enriched with long-chain *n*−3 PUFA. The incompatibility between the highly flexible, very long chain *n*−3 PUFA (EPA and DHA) and the rigid sterol moiety of cholesterol has long been recognized [45]. Studies on model membrane systems suggest that cholesterol is incompatible with a highly unsaturated lipid environment as evidenced by poor incorporation of cholesterol into DHA-containing phospholipid bilayers [46]. In addition, similar to DHA, there is evidence suggesting that other *n*−3 PUFA, for example, alpha-linolenic acid (18:3 *n*−3), may also modulate cholesterol partitioning into model membranes [47].

Changes in membrane properties due to altered lipid composition affect the organization and interactions between lipids and proteins therein. *n*−3 PUFA-induced displacement of acylated proteins from microdomains may be attributed to alterations in the lipid environment. Acylated proteins directly interact with the cytosolic leaflet of lipid microdomains by means of their saturated acyl moieties, and changes in fatty acyl composition may have a profound impact on the microdomain localization of acylated proteins [35]. Therefore, it has been proposed that altered lipid composition by *n*−3 PUFA in an otherwise highly saturated and ordered lipid microdomain is an underlying mechanism of selective protein displacement [36,38,42,48].

Alternatively, protein displacement can be attributed to changes in protein acylation by *n*−3 PUFA. Many raft proteins are modified by fatty acid acylation with myristate or palmitate. Webb et al. [37] have shown that AA, EPA and DHA can inhibit palmitoylation and that subsequent localization of Fyn to DRM was affected. The precise mechanisms underlying protein displacement by PUFA remain to be clarified, although the two above-mentioned mechanisms may not be mutually exclusive.

6. Future directions

As we expand our knowledge of principles and determinants of the lateral organization of lipid microdomains and their biological relevance, our understanding of the role and modulatory effect of *n*−3 PUFA in lipid microdomains will also significantly advance. It has been appreciated that experimental outcomes regarding biochemical isolation of microdomains vary depending on the isolation method, choice of detergent and cell type [49], suggesting the presence of heterogeneous raft populations [50]. In fact, the heterogeneity of microdomains has been increasingly appreciated with the introduction of more sophisticated techniques. If indeed such diversity of rafts exists, the nutritional data suggest that *n*−3 PUFA may also have the potential to differentially alter the lipid composition of discrete raft subspecies and their functionality. Clearly, further studies are needed to clarify the nature of lipid rafts and the influential role of different dietary *n*−6 (18:2 *n*−6; 20:4 *n*−6) and *n*−3 PUFA (18:3 *n*−3; 20:5 *n*−3; 22:6 *n*−3) family members.

7. Conclusion

Overall, these findings provide compelling evidence demonstrating that dietary sources of *n*−3 PUFA can profoundly alter the biochemical makeup of lipid rafts and caveolae membrane microdomains, thereby influencing cellular signaling (Fig. 2). These observations suggest that lipid rafts and caveolae are likely molecular targets through which long-chain *n*−3 PUFA modulate diverse biological systems and reduce the incidence and severity of human diseases.

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