The Nutritional Significance of Lipid Rafts

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Abstract

The structure, size, stability, and functionality of lipid rafts are still in debate, but recent techniques allowing direct visualization have characterized them in a wide range of cell types. Lipid rafts are potentially modifiable by diet, particularly (but not exclusively) by dietary fatty acids. However, it is not clear whether dietary polyunsaturated fatty acids (PUFAs) are incorporated into raft lipids or whether their low affinity to cholesterol disallows this and causes phase separation from rafts and displacement of raft proteins. This review examines the potential for dietary modification of raft structure and function in the immune system, brain and retinal tissue, the gut, and in cancer cells. Although there is increasing evidence to suggest that membrane microdomains, and their modulation, have an impact in health and disease, it is too early to judge whether modulation of lipid rafts is responsible for the immunomodulatory effects of n-3 PUFA. In addition to dietary fatty acids, gangliosides and cholesterol may also modulate microdomains in a number of tissues, and recent work has highlighted sphingolipids in membrane microdomains as potential targets for inhibition of tumor growth by n-3 PUFA. The roles of fatty acids and gangliosides in cognitive development, age-related cognitive decline, psychiatric disorders, and Alzheimer's disease are poorly understood and require clarification, particularly with respect to the contribution of lipid rafts. The roles of lipid rafts in cancer, in microbial pathogenesis, and in insulin resistance are only just emerging, but compelling evidence indicates the growing importance of membrane microdomains in health and disease.

Contents

INTRODUCTION

Lipid Rafts: What Are They, and Do They Actually Exist?

The lipid raft hypothesis suggests that there is a degree of self-organization within cell membranes such that dynamic microenvironments are created within the exoplasmic leaflets of the phospholipid bilayer of plasma membranes to preferentially group transmembrane proteins according to their function (71). These rafts have been proposed to serve as platforms to facilitate apical sorting, the association of signaling molecules and interactions between cell types (52, 71). It is well accepted that there are phase transitions at certain thresholds of cholesterol, giving rise to liquid-ordered and liquid-disordered phases. The liquid-ordered phases contain a high proportion of saturated fatty acids because these pack more readily against cholesterol (101). However, despite a large body of work, some doubts still persist regarding the existence and nature of lipid rafts (58, 101, 102, 137). These doubts have arisen mainly due to limitations in the interpretation of the methods available to study rafts. The most widely used technique is the preparation of detergent-resistant membranes, which are

suggested to represent raft domains, since they contain the glycosylphosphatidylinositol-anchored proteins, cholesterol, and sphingolipids characteristic of lipid rafts. In support of this idea, the liquid-ordered phases rich in cholesterol and sphingolipids in artificial membranes are resistant to detergent extraction (130). However, there is a possibility that detergent solubilization could induce nonphysiological rearrangements in bilayer structure, and in particular that the detergent could induce the formation of holes in the membrane, which allow mixing of the inner and outer leaflet and the appearance of cell-signaling proteins in detergent-resistant membrane fractions (58, 101). Clearly, direct visualization of rafts would resolve uncertainties about their existence and structure, but fluorescence microscopy studies have tended to produce mixed results (101), and it is argued that rafts are too small to be resolved by conventional microscopy techniques (137). A recent study used a combined statistical immuno-electron microscopy approach to directly visualize plasma membrane microdomains, but immuno-gold labelings of validated raft and nonraft markers were indistinguishable (22). However, statistical analysis of the plasma membrane sheets demonstrated differences in the extent of clustering tendency and size of the gold-labeled clusters. Other recently developed techniques, including stimulated emission depletion nanomicroscopy, single molecule tracing, fluorescence lifetime imaging, and fluorescence correlation spectroscopy are beginning to allow direct visualization and analysis of raft size and behavior (128). However, a recent article suggests that work in this area is becoming overly reliant on the lipid raft model and that studies are examining whether lipid rafts are involved in particular processes rather than questioning whether alternative models exist (72). Kenworthy (72) suggests that mechanistic models linking microdomain structure and function are required to systematically evaluate how the structural and dynamic features of lipid rafts influence protein diffusion and reaction kinetics.

Caveolae: A Subset of Lipid Rafts

Caveolae are a subset of lipid rafts, which are flask-shaped invaginations of plasma membrane observed by electron microscopy in many cell types, but notably not in most lymphocytes (see 55 for review). As with lipid rafts, palmitoylated proteins are responsible for the structural organization of the caveolae, forming hairpin-like structures, which tightly bind cholesterol (55). In this case, the proteins are termed caveolins and their absence from most lymphocytes suggests that they are essential for the formation of caveolae (142). Indeed, forced expression of caveolins in lymphocytes results in the formation of caveolae (42). An exception is human CD21+ and CD26+ lymphocytes isolated from peripheral blood, which stain positive for caveolin-1 (55).

Stimulation of murine macrophage cell lines with lipopolysaccharide increases expression of caveolin-1 mRNA (83), and it has been suggested that specific lipid microdomains destined to form caveolae originate in the Golgi apparatus and are transported to the plasma membrane as vesicular organelles (142). Thus it appears that the presence of caveolae at the cell surface may be a transient phenomenon, although this may depend on the cell type and/or its state of activation (55). It is interesting to note that this trafficking system operates in both directions, since caveolin can bind free cholesterol, which, if oxidized, results in the displacement of caveolin from the plasma membrane to intracellular vesicles (143).

Modification of Lipid Raft Structure and Function by Fatty Acids

Both linoleic acid and docosahexaenoic acid (DHA) increased the clustering of a lipid raft probe compared with oleic acid and untreated cells, demonstrating that polyunsaturated fatty acids (PUFAs) appear to specifically increase the clustering of proteins in cholesterol-dependent microdomains (22). The authors suggest that the poor affinity of long-chain PUFAs for cholesterol provides a lipid-driven mechanism

for lateral phase separation of cholesterol-rich microdomains and alters the dynamic partitioning of acylated proteins (22). Similarly, Shaikh et al. (132, 133) suggest that phospholipids containing highly disordered polyunsaturated acyl chains that exhibit low affinity to cholesterol would be expected to phase separate from rafts. They further demonstrated that an oleic acid (OA)-containing phosphatidylethanolamine (PE) and a DHA-containing PE phase separated differently from the lipid raft molecules, sphingomyelin, and cholesterol in monolayer and bilayer membranes (132, 133). The interactions between DHA-containing PE and cholesterol were less favorable, and as a result, these PE species were less likely to be found in detergent-resistant membrane fractions than OA-containing PE (133).

The size, stability, and functionality of putative membrane microdomains, including rafts, are still very much in debate. It has been suggested that although some domains are macroscopic and stable for extended periods, others are tiny and unstable, existing only momentarily, and as a result are very poorly understood (111, 138). A Keystone Symposium on Lipid Rafts and Cell Function proposed that membrane rafts are generally small (10–200 nm) but can coalesce through protein-protein and protein-lipid interactions to form larger platforms (112). They represent cellular structures that are potentially modifiable by diet, particularly (but not exclusively) by fatty acids. Importantly, there is increasing evidence to suggest that membrane microdomains, and their modulation, have an impact in health and disease (93, 128). This review examines the potential for dietary modification of raft structure and function in the immune system, in brain and retinal tissue, in the gut, and in cancer cells. It also examines emerging evidence for a role of lipid rafts as dietary targets in specific cells involved in insulin resistance and atherogenesis. The review mainly focuses on modification of lipid rafts by fatty acids, particularly n-3 PUFAs, although it also considers the impact of diet on sphingolipids in rafts, focusing on the important roles of gangliosides in the central nervous system, the gut, and adipose tissue.

LIPID RAFTS AND IMMUNE FUNCTION

Lipid Rafts as Platforms for Cell Activation in the Immune System

Membrane microdomains have been studied extensively with respect to T lymphocyte responses to activation (52, 71, 115). There is visual evidence of clustering of signaling components at T lymphocyte synapses using the nontoxic B subunit of cholera toxin, which binds the glycosphingolipid, GM1, a putative raft reporter (52). A novel approach whereby anti-T cell receptor (TCR)-coated microbeads were attached to T cells and then stripped away, along with patches of membrane, has demonstrated the presence of the TCR and associated signaling molecules, including linker for activation of T cells (LAT), but there did not appear to be an increase in cholesterol, as might be expected on the basis of its presence in putative lipid rafts (54). The authors concluded that protein-protein interactions, rather than protein-lipid interactions, and subsequent clustering are the key features of signaling assemblies (53). This view is supported by Douglas & Vale (33), who used sophisticated imaging techniques to track individual fluorescent proteins involved in T cell receptor signaling to show that LAT mutants lacking residues specifically required for protein-protein interactions did not cluster. Other studies by the Harder group have used fluorescence techniques to show that condensation of the plasma membrane occurs at the TCR activation site, suggesting the formation of ordered lipid phases (46, 47). These studies used a dye, Laurdan, which is incorporated into the membrane and undergoes changes in its emission spectrum, corresponding to the level of lipid ordering, allowing quantification of the condensation (46, 47). The influence of the fatty acid composition of the plasma membrane on this condensation is not clear, but it is

conceivable that increasing the polyunsaturated fatty acid content of lymphocyte plasma membranes, either in vitro or in vivo, could affect the formation of ordered lipid phases and therefore the formation of TCR-signaling assemblies.

Influence of Fatty Acids on Intracellular Signaling in T Lymphocytes; Role of Lipid Rafts

Interaction between the TCR and the antigen/MHC complex on an antigen-presenting cell induces phosphorylation of activation motifs in the accessory chains of the TcR complex by members of the Src family of tyrosine kinases, Lck and Fyn (**Figure 1**). The phosphorylated accessory chains in the TcR (CD3ζ) then activate the ZAP70 protein tyrosine kinase. Once activated, ZAP70 phosphorylates Vav, SLP76, and LAT. LAT is a key activator of downstream signaling targets, which include PLCγ, Grb2, and the small G protein, Ras. An increase in free intracellular calcium ($[Ca^{2+}]_i$) results from activation of phospholipase Cγ1 (PLCγ1). Propagation of multiple signaling pathways leads eventually to activation of transcription (**Figure 1**).

To date, most of the evidence relating to PUFA modification of lipid rafts and T cell signaling is based on detergent extraction. For example, in vitro studies using Jurkat cells have demonstrated that treatment with eicosapentaenoic acid (EPA) at a concentration of 50 μM results in marked enrichment of both EPA and

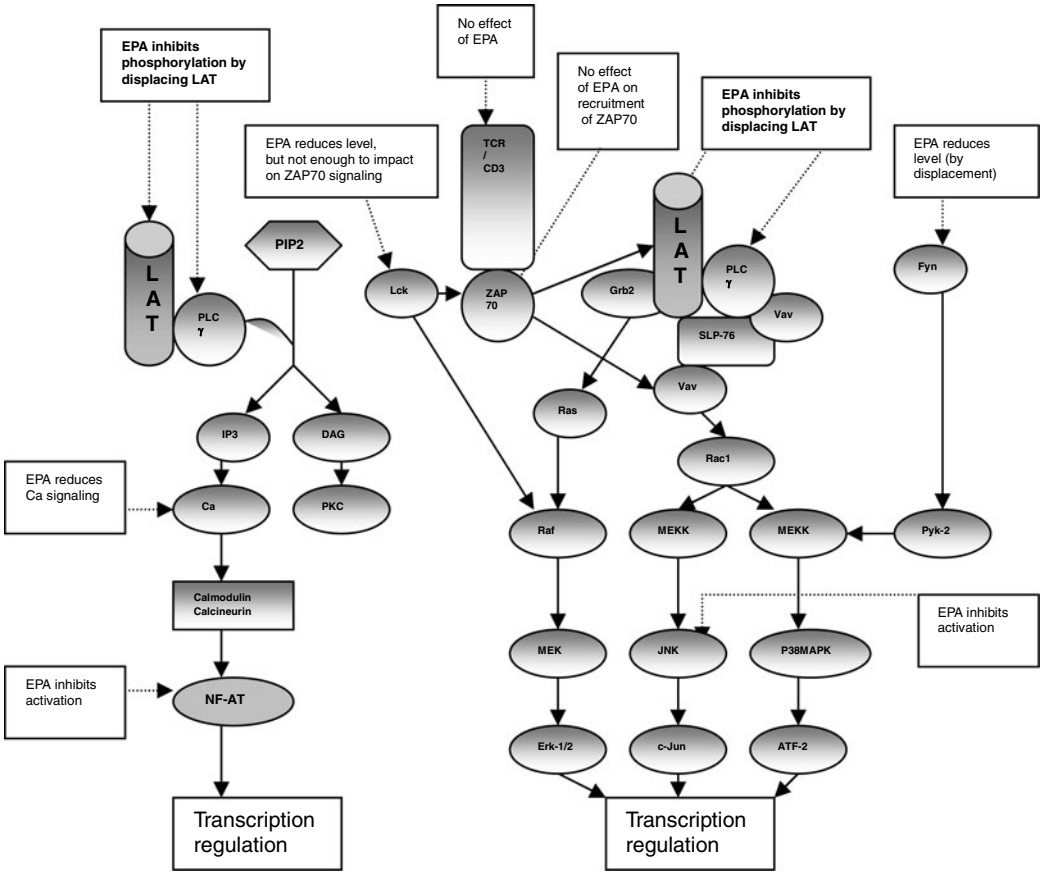

Figure 1

In vitro effects of eicosapentaenoic acid (EPA) on T lymphocyte signaling.

docosapentaenoic acid (DPA) in lipids isolated from rafts and the displacement of acylated proteins (149). There is no information regarding the extent to which membrane microdomains in human lymphocytes could be modulated by dietary supplementation with n-3 PUFAs, although lymphocyte lipids (in a whole-cell extract) are readily modified by fish oil supplementation (73), and murine T lymphocyte rafts have been shown to be responsive to dietary fish oil (38, 152). However, as discussed above, since raft formation and stability are based on interactions between saturated acyl chains and cholesterol, if detergent-resistant membrane (DRM) fractions truly represent lipid rafts, then the observations that PUFAs incorporate into these fractions is somewhat contrary to expectations.

Activation of cell signaling often involves protein palmitoylation, perhaps coupled with targeting to lipid rafts (178). Thus, altering the fatty acid composition of the cell dramatically could alter protein acylation and/or redistribution of proteins into, or out of, rafts (166). Both arachidonic acid (AA) and EPA appear to impair the formation of the immunological synapse at the T lymphocyte/antigen-presenting cell interface by inhibiting the relocalization of specific proteins to the synapse (48). Liang et al. (89) demonstrated that the Src family kinase, Fyn, could be *S*-acylated with fatty acids other than palmitate, including oleic acid and arachidonic acid, and that acylation with arachidonic acid or EPA resulted in displacement of both Fyn and LAT from membrane rafts in Jurkat cells. In agreement with this, a separate study demonstrated that DRMs from Jurkat cells treated with either AA or EPA in vitro had a reduced content of Lck and Fyn (148, 149). However, Lck-driven tyrosine phosphorylation of CD3ζ and recruitment of ZAP70 were not influenced by EPA, which led to the suggestion that the partial displacement of Lck by EPA was not sufficient to significantly perturb T cell signaling (**Figure 1**). Furthermore, Lck appears to be capable of phosphorylating CD3ζ independent of raft localization (27, 167). It has been suggested that by associating with the TCR, the transmembrane adaptor protein LAT forms a structural scaffold for subsequent signal transduction (53). Treatment of Jurkat cells and human peripheral blood T lymphocytes (PBTL) with EPA, but not stearic acid, markedly diminished the phosphorylation of LAT and PLCγ1, and it was suggested that this was due to selective partial displacement of LAT from DRMs by EPA (**Figure 1**). Together, these results suggest that tyrosine phosphorylation of LAT and PLCγ1 are the most upstream signaling events in T lymphocytes influenced by n-3 PUFAs and potentially represent a key mechanism by which n-3 PUFAs modulate lymphocyte activation. Further studies have shown that EPA treatment in vitro results in a decline in intracellular calcium signaling, which is likely to be a direct result of the effects of this fatty acid on LAT and PLCγ1 (**Figure 1**; 14, 148, 174). Separate studies have shown that exposure of murine allogeneic cytotoxic T cells to low concentrations of unsaturated fatty acids (<10 μM) inhibited the release of intracellular calcium following conjugate formation and subsequently inhibited lysis of target cells, even though binding to target cells was unaffected (5). The inhibition of calcium release was linearly correlated with a decrease in membrane acyl chain order, leading the authors to suggest that modulation of membrane structure accounted for the altered signaling and cell function (5). Only one study to date has tested the effects of dietary fish oil on early intracellular signaling in lymphocytes. In this study, feeding fish oil to rats resulted in inhibition of the tyrosine phosphorylation of PLCγ1 in spleen lymphocytes stimulated by the mitogen Concanavalin A (123).

The impact of EPA on downstream signaling events in Jurkat cells and human PBTL also appears to be selective (175). As a result of T cell activation, three families of mitogen-activated protein kinases (MAPKs) are activated by phosphorylation (**Figure 1**). The extracellular signal-regulated kinases (ERKs), c-Jun NH_2-terminal kinases (JNKs) and p38 MAPK are regulated by distinct but cross-talking signaling pathways (**Figure 1**). EPA was demonstrated to selectively block JNK activation in Jurkat

T cells compared with stearic acid (**Figure 1**; 148). In contrast, EPA did not affect activation of p38 MAPK or of ERK1/2 (175), although there is at least one report of an inhibitory effect of EPA on MAP kinase activation (89), and a separate study has reported an inhibitory effect of PUFA on ERK 1/2 phosphorylation in Jurkat cells (29). Transient transfection of Jurkat T cells with plasmids containing a reporter (luciferase) gene controlled by nuclear factor of activated T cells (NF-AT), activator protein 1 (AP-1), or nuclear factor kappa B (NFκB) demonstrated that EPA inhibited activation of NF-AT but not AP-1 or NFκB (**Figure 1**; 175). Thus, the effects of EPA on T cell signaling appear, on the whole, to be distinct and selective, at least in vitro, and to involve displacement of LAT from DRMs, impairment of PLCγ1 phosphorylation and calcium signaling, and finally, impairment of JNK and NF-AT activation (148, 149, 174, 175). It is notable that much of the work in this area has investigated the effects of EPA rather than DHA. However, one study (31) has demonstrated that phospholipase D1, which is normally located in DRMs, is displaced following treatment with DHA, and its activity is increased. Another study suggested that DHA alters the composition of membrane microdomains in Jurkat T cells and suppresses components of IL-2 receptor signaling (85).

In mice fed either fish oil or purified DHA, the recruitment of PKCθ into rafts of T cells was inhibited relative to those from mice fed corn oil, and subsequently there was reduced activation of AP-1, NFκB, IL-2 production, and T cell proliferation (37). Fish oil treatment also enhanced Fas colocalization with raft molecules, which was associated with the ability of PUFAs to promote activation-induced cell death in T cells (152).

Although a limited number of animal studies support the role of lipid rafts in mediating the effects of PUFAs on immune function, it is still largely unclear whether displacement of key signaling proteins from putative lipid rafts and the down-regulation of signaling pathways by n-3 PUFAs are physiological phenomena that could explain the immunomodulatory properties of fish oil in humans [e.g., down-regulation of the early lymphocyte activation marker, CD69 (73)]. Given the uncertainties regarding the existence and structure of lipid rafts and the interpretation of data based on differing methodologies, there is a clear need for further research to investigate the impact of modulation of plasma membrane microdomains in T cells on cell signaling and subsequent cell function.

Fatty Acid–Induced Changes in Membrane Architecture and Antigen Presentation

Studies conducted in the early to mid 1990s found that cell surface expression of major histocompatibility complex (MHC) II and antigen presentation via MHC II are decreased following in vitro exposure of antigen-presenting cells to EPA or DHA (44, 62, 74, 75, 165). A limited number of studies have investigated the effect of dietary PUFAs on MHC II expression (60, 61, 124). The study by Sanderson et al. (124) demonstrated that feeding a fish oil–rich diet to rats resulted in decreased expression of MHC II on dendritic cells, which was associated with a decreased capacity to present antigen to antigen-sensitized spleen T cells. However, the reduction in antigen presentation was probably much greater than could be explained by the reduction in MHC II expression, suggesting that other interactions between antigen-presenting cells and T lymphocytes were affected by dietary n-3 PUFAs. Recently, the effects of PUFAs on MHC I expression and on MHC I–mediated antigen presentation were reported for the first time (135). Expression of MHC I was decreased following in vitro treatment of B lymphocytes with arachidonic acid or DHA. The effect was dependent on fatty acid concentration, with arachidonic acid being slightly more effective than DHA. There were functional consequences of the reduced MHC I expression: Cytotoxic T lymphocyte–mediated lysis of target cells enriched with either arachidonic acid or DHA was decreased in a fatty

acid–concentration-dependent manner (135). By blocking resident MHC I molecules on the cell surface, it was determined that arachidonic acid and DHA decreased the surface appearance of new MHC I by slowing the flow of new class I molecules from the endoplasmic reticulum to the Golgi (135). This highlights a novel mechanism by which fatty acids affect antigen presentation, although EPA was not examined in this study, and there is, as yet, no evidence that this mechanism is reproduced in a dietary setting. In reviews by the same authors, it is suggested that PUFAs could affect MHC class I surface expression at additional points (134, 136). It is possible that PUFAs alter the conformation, lateral organization, and/or orientation of MHC class I and class II glycoproteins and of costimulatory and adhesion molecules (134, 136). Although these possibilities remain to be investigated, at least one other study supports the concept that PUFAs may influence protein trafficking: Seo et al. (131) demonstrated that DHA can inhibit trafficking of Ras and other lipidated proteins to the plasma membrane through the secretory pathway.

Fatty Acids and Potassium Channels in Lymphocytes

Voltage-gated potassium channels (K_v channels) play an important role in signaling in lymphocytes (104). The channels are transmembrane proteins, which have a nonrandom distribution in the plasma membrane (104), colocalize with CD3 molecules (104), undergo conformational changes during activation and inactivation, and can be influenced by lipid-protein interactions (70). Alteration of the lipid composition of lymphocyte membranes has been shown to alter the kinetics of the $K_v1.3$ channels of human lymphocytes: Polyunsaturated fatty acids (linoleic acid, arachidonic acid, and DHA) at 50 μM influenced both the activation and inactivation kinetics, whereas palmitic, stearic, and oleic acids did not (153). The authors speculated that this could be due to alteration of the composition of lipid rafts, within which $K_v1.3$ channels are thought to be localized, but this was not demonstrated directly and there was no information regarding the functional consequences of the altered activation kinetics of the channel (153).

Are Lipid Rafts Responsible for the Immunomodulatory Effects of n-3 Polyunsaturated Fatty Acids?

Since the 1980s, a large body of evidence has evolved that suggests that fatty acids are capable of modulating immune function (170). Initially, many of the effects were demonstrated in animals, but studies are now increasingly being conducted in either healthy humans or in patients suffering from specific immune-related diseases. However, human studies investigating the relationship between dietary fatty acids and the immune response have been disappointingly inconsistent. There are likely to be several reasons for this. First, the doses of fatty acids tested in human studies, even when administered at levels many-fold higher than normal dietary intakes, do not compare with the very high levels employed in most animal studies. Second, in studies investigating the effects of fish oil on immune function, preparations of fish oil have varied considerably in their relative contents of EPA and DHA, which might have resulted in different effects. Third, the majority of the human studies have been insufficiently powered to take into account the enormous variation in parameters of immune function, for example, ex vivo cytokine production, which is now recognized to be influenced by genotypic variation (49).

The issue of dose is clearly an important one in view of potential public health policy and recommendations. The considerable inconsistency in previous reports on the effects of n-3 PUFAs on ex vivo production of inflammatory cytokines was thought to be due to differences in administered doses (17). However, this does not fully account for the inconsistency, since some studies employing high doses of n-3 PUFAs showed no effect on cytokine production, whereas others using low doses reported inhibition (see 17 for references). Mantzioris

et al. (96) adopted the novel approach of setting target tissue concentrations of EPA, rather than target dietary intakes; they aimed to increase the mononuclear cell EPA content to 1.5% of total fatty acids by two weeks of dietary modification. Although this resulted in individual subjects consuming different quantities of n-3 PUFAs, the strategy was based on the observation by Caughey et al. (20) that the EPA content of mononuclear cells is strongly associated with ex vivo production of IL-1β and tumor necrosis factor-α (TNFα) and that 1.5% EPA results in maximum suppression of cytokine synthesis. This may explain why Thies et al. (156) reported that fish oil supplementation, providing 0.72 g/d EPA and 0.28 g/d DHA, failed to inhibit ex vivo cytokine production in healthy subjects, since the mononuclear cell EPA content only just reached 1.5% of total fatty acids. However, a study using a much higher dose of 2.1 g/d EPA plus 1.1 g/d DHA also showed no effect of fish oil supplementation on ex vivo production of cytokines, despite achieving mononuclear cell EPA levels of 2.5% of total fatty acids after 4 weeks and 3.3% at 12 weeks (172). It is likely that many of the studies described above lacked sufficient power and may therefore have missed subtle effects of the n-3 PUFAs. Thus, although the approach suggested by Mantzioris et al. (96) is interesting, it does not adequately explain the discrepancies in the literature. Clarification is still required, perhaps through the design of adequately powered studies using a range of doses of n-3 PUFAs and assessment of mononuclear cell EPA content. The attainment of a notional threshold of 1.5% EPA should perhaps be revisited and considered in the context of changes in membrane microdomains, which could then be mapped onto cellular functions.

It is notable in some studies that EPA and DHA have differential effects on immune function. For example, Thies et al. (158) demonstrated that fish oil, but not DHA, inhibited natural killer cell activity, suggesting that EPA rather than DHA might be responsible for the effect. However, Kew et al. (73) presented evidence to suggest that DHA altered immune function to a greater degree than did EPA. The issue of the potentially different immunomodulatory properties of EPA and DHA is one which has not been adequately investigated and is clearly important given the variation in the proportions of EPA to DHA in preparations used in the studies described above. There is a need, therefore, not only to examine the effects of the dose of n-3 PUFAs on immune and inflammatory responses, but also the separate effects of EPA and DHA and their combination in different ratios. If these fatty acids truly possess different immunomodulatory capabilities, a mechanistic basis needs to be derived, and this may or may not involve effects at the membrane microdomain level.

It is apparent from the literature that fish oil has a greater impact on immune function in the elderly compared with young subjects (100, 156–158). The mechanistic basis for this is not understood, but if fatty acids modulate immune function by raft-dependent means, then an explanation is required for the differential response of young versus older subjects. Thus, although some compelling evidence from in vitro and animal experiments indicates that n-3 PUFAs modulate immune function by effects on membrane microdomains, there is a lack of direct evidence that this is physiologically relevant in humans, and a number of aspects of the immunomodulatory effects of n-3 PUFA are difficult to explain on the basis of lipid rafts at this stage.

NUTRITIONAL SIGNIFICANCE OF LIPID RAFTS IN VISUAL AND COGNITIVE FUNCTION

Polyunsaturated Fatty Acids, Lipid Rafts, and Brain Function

The fatty acid composition of central nervous tissue of vertebrates plays an important role in maintaining the structural and functional integrity of membranes. When levels of DHA are reduced in animals, they underperform in learning and cognitive function tests, but this can be reversed by restoring DHA

supply (9, 10). The mechanisms by which DHA-containing phospholipids could affect cognitive function are uncertain, but several have been suggested (122). Among its many roles, DHA influences the function of the blood-brain barrier (122), the activity of membrane-bound enzymes (15) and ionic channels (163), dopaminergic and serotoninergic neurotransmission (79, 177), and signal transduction (160). In addition, DHA can protect against neuronal cell death by apoptosis (2), induce synaptic growth cones during neuronal development (8, 12), regulate nerve growth factors (63) and neuronal size (1), and modulate gene expression in the brain (9, 10, 30, 78). Whether these effects are manifested through influence on the structure or biophysical properties of membrane microdomains or lipid rafts is as yet relatively unexplored. However, caveolae are widely expressed in the central nervous system in brain microvessels, endothelial cells astrocytes, oligodendrocytes, Schwann cells, dorsal root ganglia, and hippocampal neurons (3). They are absent from most neurons and neuroblastoma cells, but these appear to possess planar lipid rafts instead (3). It has been proposed that neurotransmitter signaling may occur through clustering of receptors in lipid rafts or caveolae, and the effects of lipid rafts on neurotransmitter signaling have been implicated in neurological and psychiatric diseases (3). However, there is currently no clear definition of the role of rafts in neurotransmitter signaling, and there are significant limitations in our understanding of their role in normal development and disease.

Polyunsaturated Fatty Acids in Visual and Cognitive Function During Early Development and Age-Related Degeneration

Because the incorporation of arachidonic acid and DHA is particularly high during the prenatal period and the first two years of life (36), there has been significant interest in the possibility that supplementation during pregnancy and lactation, or the addition of PUFAs to infant formulas, may have an impact on visual and cognitive development. Kitajka et al. (77, 78) demonstrated that DHA-containing phosphatidylethanolamine species (particularly 18:0/22:6) in rat brain were increased after consumption of α-linolenic acid or a mixture of EPA and DHA from conception to adulthood, indicating that it is possible to increase levels of DHA in neural membranes. However, despite evidence for diverse effects of PUFAs in neuronal function, as described above, evidence for effects of n-3 PUFA supplementation on visual and cognitive development during childhood is still rather preliminary. Several reviews, including two Cochrane reviews, conclude that evidence for beneficial effects of n-3 PUFA supplementation on early visual and cognitive development in term and preterm infants is inconclusive (36, 139, 140) and too inconsistent to justify the supplementation of infant formula with DHA (99). However, the term "cognition" is very broad, covering memory, learning, reasoning, attention, and language, and it is clearly difficult to compare individual studies that assessed visual and cognitive outcomes in different ways and in different study designs. Thus, there is scope for further investigation in this area. The potential benefits of DHA supplementation on cognition in older children has not yet been investigated, but the potential for benefit traditionally has been believed to be smaller than that for young infants because DHA incorporation becomes less significant after two years of age (110). However, it has been suggested that deficiencies or imbalances in long-chain PUFAs may be associated with childhood developmental and psychiatric disorders, including attention-deficit hyperactivity disorder, dyslexia, dyspraxia, and autism spectrum disorders (6, 116). Attempts to treat these conditions with PUFA supplementation have been described in a small number of studies, but these have involved different populations, study designs, treatments, and outcomes, and the results have been mixed (4, 116). An example is the Oxford-Durham study, a double-blind, randomized, placebo-controlled study with a one-way crossover (placebo to active

treatment), which reported improvements in reading, spelling, and behavior following supplementation with EPA, DHA, and γ-linolenic acid in children with developmental coordination disorder, a condition affecting approximately 5% of school-age children (117). The high proportion of EPA in the supplements, relative to DHA, as well as the inclusion of the n-6 PUFA, γ-linolenic acid, make the interpretation of these results somewhat difficult, as the improvements cannot be clearly ascribed to a single fatty acid. Although it might be predicted that DHA would be the primary component of interest, intervention studies in adult psychiatric disorders have also reported some beneficial effects of EPA-rich supplements (147). Indeed, it has recently been suggested that "EPA may prove more efficacious in addressing nervous system disorders, such as depression, while DHA may be more beneficial in heart disease prevention" (18). This has led to the suggestion that the influence of n-3 PUFAs might be manifested through cerebrovascular effects rather than (or in addition to) the incorporation of DHA into structural lipids (potentially in lipid rafts) in the brain (147). However, this suggestion is as yet unsubstantiated.

Epidemiological associations between fatty acid abnormalities and adult psychiatric disorders, such as depression and schizophrenia, have been reported in the literature (see 147). Studies investigating the therapeutic potential of n-3 PUFAs in mental illness are as yet inconclusive, but recent reviews suggest that the available evidence is strong enough to justify continued study (7, 16, 92, 147). There has also been considerable recent interest in the possible role of dietary fatty acids in age-related cognitive decline and in cognitive impairment related to Alzheimer's disease (AD). Several observational and epidemiological studies suggest that saturated fatty acids have negative effects on cognitive function and that high intakes of MUFAs and PUFAs are associated with reduced risk for cognitive decline and protection against the risk of AD (144). However, a Cochrane review (91) of the evidence that n-3 PUFA supplementation prevents cognitive impairment and dementia in cognitively intact elderly persons found that no randomized trials in their search met the selection criteria (placebo-controlled, double-blinded, minimum six months duration, involving persons aged 60 and over without pre-existing dementia, and employing cognitive endpoints). They concluded that although a growing body of evidence from biological, observational, and epidemiological studies suggests that n-3 PUFAs provide a protective effect against dementia, data from appropriate randomized trials are required to support this. These data are just beginning to emerge, although results from a recent trial showed no effect of 26 weeks of n-3 PUFA supplementation on cognitive performance in older subjects without dementia (161).

In addition to the observation that aging has been associated with the loss of both arachidonic acid and DHA in the brain (28, 40), some evidence from postmortem analysis indicates that brain membrane phospholipids from AD patients are deficient in DHA (113). Hashimoto et al. (56) investigated the effects of dietary DHA on levels of β-amyloid (Aβ) and on cholesterol in DRMs from the cerebral cortex in a rat model of AD and found that both were decreased compared with controls. They also reported a negative correlation between the number of reference memory errors and the molar ratio of DHA to palmitic and stearic acids, which led them to suggest that DHA protected against memory deficits in this model (56). In a later study, they related these effects to changes in synaptic plasma membrane fluidity (57).

Dietary Gangliosides, Polyunsaturated Fatty Acids, and Retinal Development and Function

Dietary long-chain PUFAs have long been recognized to modify the fatty acid composition of phospholipids in the developing retina (150, 151). Gangliosides are sialic acid–containing glycosphingolipids that are present in the outer leaflets of all mammalian plasma membranes, where they participate in recognition and

signaling activities. They are enriched in lipid rafts in neuronal cell membranes and play an important role in the retina as well as in brain physiology and pathology (13, 145). Because neonates and infants receive gangliosides from human milk, it has been suggested that dietary gangliosides may be important for retinal development. Rats fed dietary gangliosides showed increased total retinal ganglioside content during development; the compositional changes in retinal phospholipids in response to both dietary gangliosides or PUFAs may affect light adaptation and signaling, which in turn could lead to enhanced retinal development in neonates (105).

In contrast to these important roles of neuronal gangliosides, it has been reported that brain injury can lead to the release of gangliosides from damaged neuronal cells and potentially can induce the production of inflammatory mediators by brain microglia and astrocytes (76, 114) by a mechanism that involves signaling through Toll-like receptor 4 (67).

Gangliosides have also been reported to play a role in the extracellular deposition of Aβ, which is a hallmark of AD (173). Complex glycosphingolipids appear to mediate transport of amyloid precursor protein to the cell surface, which leads to proteolytic processing and formation (173). Sphingomyelin is a major source of ceramides, which are lipid mediators generated by sphingomyelinases following activation by inflammatory cytokines (80) and oxidative stress (25). Cutler et al. (25) reported significant increases in levels of membrane-associated oxidative stress, long-chain ceramides, and free cholesterol in brain cells during normal aging in mice, in AD patients, and in neurons exposed to Aβ. Treatment of neurons with α-tocopherol or an inhibitor of sphingomyelin synthesis prevented these changes and protected against Aβ-induced death (25). The dietary implications of these findings remain to be determined.

Cholesterol and Neuronal Function

The physiological role of cholesterol in the brain is unclear, although it appears to play important roles in regulating synaptic plasticity, memory encoding, and storage (98, 146). Both local synthesis and uptake are thought to contribute to the brain pool of cholesterol, and, as with DHA feeding (10), a cholesterol-enriched diet improved spatial learning in the Morris water-maze paradigm (34). These behavioral changes were shown to be associated with modulation of both pre- and postsynaptic function (34). Although a deficit in brain cholesterol appears to be detrimental for brain function, a body of evidence suggests that a reduction in brain cholesterol could be beneficial in preventing the accumulation of Aβ and therefore could be beneficial in the prevention and cure of AD (see 82). In accordance with this finding, patients treated with statins have a reduced incidence of AD (65), although this could arguably be due to effects of statins that are independent of their cholesterol-lowering ability, such as anti-inflammatory activity (82). Nevertheless, in vitro studies in cells overexpressing amyloid precursor protein or other proteins involved in Aβ accumulation also show that cholesterol synthesis inhibitors and statins inhibit Aβ release (23, 35, 39). This led to the conclusion that excess amyloid in AD is caused by high cholesterol and that Aβ is produced in cholesterol-rich lipid rafts (82). However, this conclusion is controversial because some of the cholesterol-reduction treatments resulted in a very high membrane cholesterol loss, which might be expected to disrupt not only lipid rafts, but also the membrane as a whole (82). Furthermore, different extraction and separation protocols for lipid rafts resulted in different conclusions, and several studies show only minor quantities of Aβ or its precursor protein in rafts (35). Opinion clearly differs on the issue, but Ledesma & Dotti (82) conclude that the data do not prove that rafts and high neuronal cholesterol are involved in Aβ accumulation in AD, while recognizing that future work may clarify the issue. In the meantime, there are studies demonstrating both a reduced (81) and elevated (129) level of cholesterol in AD brains. It is also not clear at present whether extracellular uptake of cholesterol is a significant

source of neuronal cholesterol and, in an argument against a useful role for diet or statins in brain function, it has been proposed that neuronal cholesterol content is chiefly dependent on internal synthesis (32).

LIPID RAFTS AND CANCER

Elevated levels of saturated fatty acids and cholesterol are thought to be a general property of cancerous cells (88, 138). The increased cholesterol, often caused by upregulation of hydroxymethylglutaryl-coenzyme A (HMG CoA) reductase, is suggested to lead to coalescence of lipid rafts and thus to stimulate the oncogenic pathway (88). Cholesterol depletion disrupts rafts, bringing about a redistribution of raft components, and these cholesterol-depleted cells appear to be particularly sensitive to apoptosis (88). However, the effects of cholesterol depletion are nonspecific and disrupt many cellular processes, which limits their application in therapy. If dietary components, such as n-3 PUFAs, were to bring about a more targeted reduction in raft cholesterol content, they could prove useful. Interestingly, n-3 PUFAs are reported to suppress HMG CoA reductase activity (84), and some evidence suggests that n-3 PUFAs have anticarcinogenic properties (45, 66, 125).

A number of lipid raft proteins have been postulated to be targets for the effects of n-3 PUFAs (see 138 for review). These include estrogen receptors and epidermal growth factor receptors (EGFRs), which colocalize in rafts (97). N-3 PUFAs have been shown to modulate signaling through these receptors and to inhibit tumor growth in breast cancer cell lines and models (125). A specific role for lipid rafts was suggested by Schley et al. (126), who demonstrated that EPA and DHA were incorporated into lipid rafts (prepared by detergent extraction) in MDA-MB-231 cells, replacing sphingomyelin, cholesterol, and diacylglycerol, decreasing EGFR levels in the rafts, and increasing the phosphorylation of both EGFR and p38 MAPK. The sustained activation of EGFR and p38 MAPK was associated with a decrease in growth rate (126) and has also been implicated in the induction of apoptosis (24, 127). The overall picture presented by Schley et al. (126, 127) is that n-3 PUFAs are incorporated into DRMs of cultured breast cancer cells, where they activate neutral sphingomyelinase, which results in depletion of membrane sphingomyelin and cholesterol exclusion. Sphingomyelin hydrolysis generates ceramide, a proapoptotic signal, whereas the lowered cholesterol content results in cytosolic expulsion of EGFR. Subsequent phosphorylation of this receptor generates further proapoptotic signals, perhaps through p38 MAPK activation. Ultimately, these events lead to cell apoptosis and reduced cell numbers. This work highlights membrane microdomains as the focus for improved understanding of membrane-mediated signaling events and, in particular, a novel, membrane-based mechanism for the inhibition of breast tumor growth by n-3 PUFAs. However, it would be important to confirm the physiological application of the findings, given the relatively high concentrations of total fatty acids used (100–150 μM) and the use of detergent for the characterization of lipid rafts.

Several other raft-associated molecules have also been implicated as potential targets for modulation by n-3 PUFAs. For example, cell adhesion molecules involved in metastasis are often located within lipid rafts, and their expression and signaling are affected by the cellular cholesterol or n-3 PUFA content. However, there is as yet no direct evidence that this involves redistribution or displacement of raft proteins (138). It is also worth putting these observations in the context of the total evidence for the protective effects of n-3 PUFAs in breast cancer. Although some epidemiological studies suggest an inverse relationship (118), prospective cohort studies do not, on the whole, support an association between n-3 PUFA consumption and breast cancer risk (94). Nevertheless, two recent reviews explore potential lipid raft–mediated mechanisms for modulation of signaling pathways by fatty acids in cancer (21, 109). Sphingolipids are also receiving increasing attention in the context of cancer research on the

basis of their roles in the regulation of proliferation, apoptosis, transformation, differentiation, and motility (103, 173). As described above, alterations in sphingolipid metabolism have already been shown to contribute to the development of chemoresistance, and they therefore represent a potentially important target for future therapeutic strategies, which may well exploit their location in lipid rafts.

LIPID RAFTS IN THE GUT

Glycolipids appear to be the predominant raft-promoting components of the small intestinal brush border, constituting up to 30% of total lipid, whereas cholesterol and sphingomyelin make up only about 10% and 5%, respectively (50). It is suggested to be because of this make-up that lipid rafts from small intestinal brush borders are largely resistant to cholesterol depletion (26, 50). As with other cell types, interpretation of data concerning lipid rafts in the small intestine is largely indirect and subject to uncertainty. However, Danielsen & Hansen (26) maintain that the lipid rafts in the small intestinal brush border are stable rather than transient and may be larger than those in other cell types. They also suggest that organization into glycolipid-based microdomains, stably cross-linked by galectin-4, plays a role in apical trafficking (51). On the other hand, lipid rafts (and in particular, sphingolipid-enriched microdomains) have been demonstrated to be exploited as a portal for the entry of pathogens in epithelial cells (reviewed in 11, 95, 173). In particular, cell surface gangliosides have been identified as unintended target receptors for bacterial adhesion (119). It has been suggested that dietary gangliosides could act as putative decoys and thus interfere with pathogen entry (119). The role of gangliosides in human breast milk is an active area of research, particularly with respect to early infant development and gastrointestinal infections. Differences in the relative concentration of individual gangliosides in human milk from mothers delivering preterm and term infants have been reported (120). One study has tested the impact of infant formula containing gangliosides on the fecal flora of preterm infants and demonstrated lower *Escherichia coli* counts and higher levels of the beneficial *Bifidobacteria* compared with standard formula (121). However, whether these effects in any way involve modulation of cell composition or behavior by gangliosides is not known; it is notable that the carbohydrate portions resemble those of free oligosaccharides with proven prebiotic capabilities (119). Nevertheless, dietary gangliosides have been shown to modify the composition of the brush border membrane in intestinal mucosa (106, 107), and an increase in ganglioside content decreased the cholesterol and caveolin-1 content of lipid microdomains (108). Cholesterol depletion in membranes inhibits cellular entry of pathogens and generation of inflammatory signals by disrupting microdomain structure; thus, the authors went on to demonstrate that feeding dietary gangliosides resulted in reduced generation of inflammatory eicosanoids and cytokines by intestinal mucosa following acute exposure to bacterial endotoxin (108).

LIPID RAFTS, DIABETES, AND CARDIOVASCULAR DISEASE

Membrane Microdomains, Gangliosides, and Insulin Sensitivity

Recent evidence has highlighted the possibility that obesity and insulin resistance involve disordered lipid dynamics and membrane microdomains (43). Studies have identified the presence of a caveolin-binding domain in the insulin receptor and direct binding of the insulin receptor and caveolin-1 (43). As discussed in this review, gangliosides, which are thought to be key components of lipid rafts, play important roles in both the brain and the small intestine. Recent studies have described the generation of mutant mice unable to synthesize the widely distributed ganglioside, GM3: the mice, although viable, demonstrate a heightened sensitivity to insulin (169). Obese Zucker *fa/fa* rats and *ob/ob* mice have increased levels of GM3 synthase mRNA in their adipose tissue

(64), and direct addition of GM3 to 3T3-LI adipocytes suppresses insulin-stimulated phosphorylation of the insulin receptor (154), supporting a regulatory role of the receptor by this and perhaps other gangliosides. Insulin signaling is in fact initiated in glycosphingolipid-enriched membrane domains (both rafts and caveolae) (64). However, in the GM3 synthase knockout mice, insulin receptor phosphorylation was altered in skeletal muscle and liver, but not in adipose tissue, leading the authors to suggest that this may reflect differences in the glycolipid composition or signaling pathways in the two tissues (169). The nutritional significance of the absence of GM3 was highlighted by the fact that lack of GM3 conferred protection from high-fat-diet-induced insulin resistance; after the high-fat dietary treatment, the mutant mice had lower insulin levels and enhanced responsiveness to glucose in comparison with wild-type mice (169). It should be noted that although the authors refer to the mutants as having heightened insulin sensitivity, there were no statistically significant differences in insulin sensitivity measured by euglycemic-hyperinsulinemic clamps; the differences were only apparent for the glucose tolerance tests and measurements of insulin receptor phosphorylation (169). Nevertheless, this study provides an intriguing insight into the possibility that the GM3 ganglioside is involved in diet-induced insulin resistance in the metabolic syndrome. Furthermore, in vitro studies have shown that the insulin receptor forms complexes with GM3 and with caveolin-1 in an independent manner and that GM3-enrichment of membranes results in increased mobility of the insulin receptor by weakening its interaction with caveolin-1 (69). These studies further identified that a lysine residue located just above the transmembrane domain of the insulin receptor β subunit was essential for the interaction with GM3 (69). Together, the data suggest that GM3 regulates the localization of the insulin receptor in membrane microdomains. However, Kabayama et al. (69) further proposed that separate and distinct membrane subdomains comprise insulin-receptor/caveolin complexes and insulin receptor/GM3 complexes; they term the former caveolae and the latter glycosphingolipid-enriched domains (GEMs). Live cell studies of the real time lateral interactions between the insulin receptor, caveolin-1, and GM3 showed that accumulation of GM3 appears to cause displacement of the insulin receptor from the caveolae microdomains into GEMs, and the change in ratio of insulin receptor complexes in caveolae versus GEMs alters insulin signaling in adipocytes (69). There is some evidence that the insulin receptor is not alone in being regulated by its interactions with GM3; Kabayama et al. (69) suggest that the same concepts for control may be applied to receptor tyrosine kinases.

In vitro studies demonstrate that in adipocytes in which insulin resistance was induced by TNF-α, inhibition of insulin signaling and the elimination of insulin receptors from caveolae were associated with accumulation of GM3 (68, 69, 154). Conversely, GM3 depletion counteracted the inhibitory effects of TNF-α on insulin receptor internalization and elimination from caveolae (64, 68, 69, 154). This suggests that the link between chronic low-grade inflammation in obesity and metabolic syndrome might directly involve gangliosides in a membrane microdomain-related mechanism.

A recent study demonstrates that a synthetic inhibitor of glycosphingolipid synthesis can improve glucose control and insulin sensitivity in both Zucker diabetic rats and diet-induced obese mice, raising the possibility for new drug-based treatments of type 2 diabetes (176). However, since the inhibitor acts at the initial step in the synthesis of a large number of glycosphingolipids, the identity of the critical component is not clear (176).

Modification of Endothelial Nitric Oxide Synthase via Membrane Microdomains

Endothelial nitric oxide synthase (eNOS) plays an important role in regulation of vascular tone by nitric oxide (NO), and this has significant

implications for cardiovascular disease (162). The enzyme is thought to be located in caveolae (41, 168) and its activity regulated by calmodulin, which dissociates it from caveolin-1 (155). Given the substantial evidence for cardioprotective effects of n-3 PUFAs through a number of mechanisms, their impact on NO production and regulation of eNOS activity has been of interest. Li et al. (86, 87) reported that in vitro treatment of human umbilical vein endothelial cells with both EPA (86) and DHA (87) altered the phospholipid composition of caveolae and displaced caveolin-1 from microdomains. These effects were accompanied by translocation of eNOS from caveolae to soluble membrane fractions and an increase in eNOS activity (86, 87). The physiological relevance of these findings remains to be confirmed.

Fatty Acids, Lipid Rafts, and Environmental Toxicity in Endothelial Cells

The potential for nutrition to modulate the toxicity of environmental pollutants is becoming increasingly recognized. Because many persistent organic pollutants are fat-soluble, it has been proposed that high-fat foods, which contribute a potential excess of calories as well as environmental toxins, pose multiple risks to health. The uptake of polychlorinated biphenyls (PCBs) by endothelial cells has been shown to be increased in the presence of linoleic acid (141), and it has been suggested that PCBs activate endothelial cells by mechanisms involving caveolae (90). It is possible that PCBs accumulate in the caveolae of endothelial cells and somehow interact with specific fatty acids since linoleic acid amplified, whereas α-linolenic acid protected against, PCB-induced endothelial activation (159, 164). Oily fish containing long-chain n-3 PUFAs could potentially be contaminated with organic pollutants and thus represent a food group that has substantial health benefits as well as possible risks (59). However, the consensus is that fish consumption should be encouraged because of its considerable cardioprotective properties.

CONCLUSIONS

Improved techniques for direct visualization of putative lipid rafts are beginning to resolve the uncertainty about their existence and structure, but the extent to which they are modifiable by diet remains to be clarified. In vitro studies suggest that PUFAs cause substantial reorganization of membrane microdomains, which has an impact on signaling and function in a number of cell types. The relevance of this work to dietary modification of cell function requires further investigation. Gangliosides are emerging as raft components with diverse roles in several tissues, including the brain, small intestine, and adipose tissue. In each of these tissues, they are critical for normal development and function, but they also appear to be involved in pathological processes. The potential for dietary modification of gangliosides is relatively unexplored.

SUMMARY POINTS

1. Lipid rafts are potentially modifiable by diet, particularly (but not exclusively) by dietary fatty acids. However, it is not clear whether dietary PUFAs are incorporated into raft lipids or whether their low affinity to cholesterol disallows this and causes phase separation from rafts and displacement of raft proteins. Incorporation of PUFAs into rafts is somewhat contrary to expectations, but most of the evidence suggesting this comes from in vitro experiments and remains to be clarified.

2. In vitro and animal studies suggest that n-3 PUFAs modulate the structure and composition of lipid rafts, displace key signaling proteins, and alter protein trafficking in T lymphocytes. The physiological relevance of these observations needs to be examined in vivo in humans in order to judge whether modulation of lipid rafts is responsible for the immunomodulatory effects of n-3 PUFAs.
3. Animal studies suggest that brain DHA is important for learning and cognitive function. Evidence for beneficial effects of n-3 PUFA supplementation in visual and cognitive function in infants and children is inconclusive, and although it might be predicted that DHA would most likely be beneficial on the basis of its abundance in the central nervous system, some evidence suggests that EPA is efficacious and the effects do not necessarily involve incorporation into structural lipids or rafts in the brain.
4. Gangliosides are enriched in lipid rafts in neuronal cell membranes, but their roles are complex. They are important for retinal and brain development, but they also appear to play a role in the inflammatory response to brain injury and in AD. Dietary PUFAs, gangliosides, and cholesterol may influence these processes, but this area is poorly understood.
5. Exposure of specific cancer cell lines to n-3 PUFAs results in disruption of lipid rafts, generation of ceramide from sphingomyelin hydrolysis, expulsion of the EGFR, and induction of apoptosis. This highlights membrane microdomains as potential targets for inhibition of tumor growth by n-3 PUFAs. Sphingolipid metabolism appears to contribute to chemoresistance and may represent a potentially important target for future therapeutic strategies.
6. Glycolipids are prominent raft-promoting components of the small intestinal brush border and have been suggested to play a role in apical trafficking. However, they can also be exploited as a portal for the entry of pathogens. Dietary gangliosides could act as decoys to interfere with pathogen entry; this could represent a mechanism by which gangliosides from breast milk protect against gastrointestinal infections in infants.
7. In addition to the roles in the brain and small intestine, both desirable and undesirable, gangliosides may also play a role in the induction of insulin resistance in adipose tissue. Insulin signaling is initiated in glycosphingolipid-enriched membrane domains and the ganglioside, GM3, appears to mediate the high-fat-diet-induced insulin resistance in an animal model by direct effects on the insulin receptor.
8. Preliminary evidence suggests that long-chain n-3 PUFAs may have an impact on endothelial function through mechanisms involving membrane microdomains.

FUTURE ISSUES

1. One of the problems in this area has been that different methodologies generate membrane material with differing structural characteristics, making interpretation difficult (171). Improved techniques for the visualization of lipid rafts in intact living cells will, without doubt, aid advances in our understanding of the true nature of lipid rafts.

2. It will be particularly important to clarify the impact of dietary fatty acids on lipid raft structure, using direct techniques, and to examine whether modulation of lipid rafts is responsible for the immunomodulatory effects of n-3 PUFAs.
3. The roles of fatty acids and gangliosides in cognitive development, age-related cognitive decline, psychiatric disorders, and AD are poorly understood and require clarification, particularly with respect to the contribution of lipid rafts.
4. The roles of lipid rafts in cancer, in microbial pathogenesis, and in insulin resistance are only just emerging, but compelling evidence indicates the growing importance of membrane microdomains in health and disease.
5. Future work should advance our understanding of the mechanisms by which dietary components, particularly fatty acids, influence the activity of so many different cell types and with such diverse implications for normal development, health, and disease.

DISCLOSURE STATEMENT

The author is not aware of any biases that might be perceived as affecting the objectivity of this review.

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Contents

Indexes

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