Review

Dietary Fatty Acids and Membrane Protein Function

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In recent years, there has been growing public awareness of the potential health benefits of dietary fatty acids, and of the distinction between the effects of the ω6 and ω3 polyunsaturated fatty acids that are concentrated in vegetable and fish oils, respectively. A part of the biologic effectiveness of the two families of polyunsaturated fatty acids resides in their relative roles as precursors of the eicosanoids. However, we are also beginning to appreciate that as the major components of the hydrophobic core of the membrane bilayer, they can interact with and directly influence the functioning of select integral membrane proteins. Among the most important of these are the enzymes, receptors, and ion channels that are situated in the plasma membrane of the cell, since they carry out the communication and homeostatic processes that are necessary for normal cell function. This review examines current information regarding the effects of diet-induced changes in plasma membrane fatty acid composition on several specific enzymes (adenylate cyclase, 5'-nucleotidase, Na^+/K^+-ATPase) and cell-surface receptors (opiate, adrenergic, insulin). Dietary manipulation studies have demonstrated a sensitivity of each to a fatty acid environment that is variably dependent on the nature of the fatty acid(s) and/or source of the membrane. The molecular mechanisms appear to involve fatty acid-dependent effects on protein conformation, on the "fluidity" and/or thickness of the membrane, or on protein synthesis. Together, the results of these studies reinforce the concept that dietary fats have the potential to regulate physiologic function and to further our understanding of how this occurs at a membrane level.

Keywords: dietary fatty acids, adenylate cyclase, Na^+/K^+ ATPase, 5'-nucleotidase, adrenergic receptors, insulin receptors, opiate receptors.

Introduction

One of the most intriguing and complex questions confronting lipid biochemists is that of the nature of the relationship between membrane fatty acids, particularly the essential polyunsaturated fatty acids (PUFAs), and the integral proteins that determine the functional properties of a membrane. Earlier concepts held that the acyl constituents esterified to complex lipids were merely the "molecular cement" which bonded together the lipid bilayers. More recently, we have begun to believe that at least part of the essentiality of the PUFAs resides in their requirement for a direct modulation of membrane protein function, via mechanisms that are distinct from their obvious role as precursors of the bioactive eicosanoids.[1] A regulatory function for fatty acids is suggested in part by the dramatic heterogeneity and selectivity in their tissue/membrane distribution. The fatty acid profiles of complex lipids vary with the type of lipid, position within a phospholipid, body organ, organ region, and cell type, and, in some cases (e.g., retina, brain), even to selective domains within the plasma membrane.[2-5] On physiologic/behavioral levels, the importance of PUFAs in membrane function has been reinforced by observations that their deficiencies in brain and retinal tissues can result in compromised visual acuity[4] and learning ability,[6-9] and even in neurologic problems.[10] However, to provide other than circumstantial evidence of the importance of membrane fatty acids requires that their effects on specific protein functions be identified and characterized at a molecular/cellular level. Ultimately, it will be necessary to elucidate the mechanistic basis for these effects. Most of the initial studies of the relationship between membrane structure and function involved examining the effects of perturbing the membrane lipids using degradative techniques such as phospholipase digestion, detergent treatment, insertion of "fluidizing" fatty acids, and introduction

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of lipophylic agents such as polyene antibiotics.[11-14] Other investigators have approached this problem by reconstituting membrane proteins into artificial phospholipid bilayers[15] or by preincubating membranes with free fatty acids for short periods of time.[16-18] The results of each of these studies supported the hypothesis that the lipid bilayer was important in protein function; however, each had the disadvantage of involving nonphysiologic conditions, making it difficult to assess their functional significance. For example, in acute exposure experiments, the acids have a relatively loose association with the membrane, and tend to be intercalated into the hydrophobic regions to produce significant changes in the packing of the lipid molecules.[19] This does not reflect the situation *in vivo*, in which most acyl groups are esterified to complex lipids.[3] Only in recent years have investigators begun to take full advantage of dietary studies to examine the relationship between membrane lipids and protein function. These studies, in which animals or cultured cells are given diets (or supplements) that are controlled with respect to their fatty acid composition, have the distinct advantage of the fact that the membranes are altered biosynthetically. Under these more physiologically relevant conditions, we have seen that the functioning of a select group of membrane proteins are sensitive to alterations in the fatty acid environment.

The purpose of this paper is to review recent information concerning the effects of dietary fatty acids on the functioning of several enzymes and receptor complexes that are localized in the plasma membrane. From this information, an attempt shall be made to determine whether there is sufficient evidence, based on fatty acid, protein, and/or tissue specificity, to support the hypothesis that membrane fatty acids have important, non-eicosanoid regulatory functions. For previous reviews of the effects of fatty acids (and lipids in general) on protein function, the reader is referred to the following reports: Yeagle,[20] Merrill,[21] Sandermann,[22] Brenner,[23] Stubbs and Smith,[24] Spector and Yorek,[25] Brenner et al.,[26] and Carruthers and Melchior.[27]

Modification of membrane polyunsaturated fatty acid composition

Changes in dietary fat can lead to marked changes in the fatty acid composition of membrane phospholipids. The resulting profiles are the products of a complex series of competitions between the many enzymes that desaturate, elongate, activate, and esterify the acyl chains. An in-depth discussion of this topic is beyond the scope of this article; however, there are several points that should be reiterated briefly as background.

The major essential PUFAs, arachidonate (C20:4ω6) and docosahexaenoate (C22:6ω3), are either ingested *per se* from the diet, or are derived from their precursors, linoleic (18:2ω6) or linolenic (18:3ω3) acids. The dietary sources of these acids and their metabolic pathways have been recently reviewed.[4,28,29] The patterns of distribution and amounts of the ω6 and ω3 PUFAs vary widely between body tissues. The ω6 species are the major PUFAs in most peripheral tissues (e.g., heart, liver), and they are also present in large amounts in nervous tissues. The ω3 series are concentrated in the outer segments of photoreceptor cells of the retina, in sperm, and in synaptic, mitochondrial, and microsomal membranes of the cerebral cortex.[30,31] An even greater specificity of localization was recently suggested by Salem et al.[30] when they proposed that both classes of PUFAs are preferentially localized on the cytosolic leaflet of the plasma membrane and around membrane-bound proteins. Most of the PUFAs that are associated with the membrane are esterified at the 2 position of phospholipids; the small but significant amounts that are found free in the membrane are probably involved in "second messenger" functions, either as the PUFAs *per se* or as their eicosanoid derivatives. It has been recently shown that the two families of PUFAs can also produce very long (C_{24} to C_{36}) polyenoic fatty acids that are esterified at the 1 position of phosphatidylcholine in the retina[31] and brain,[32] although the function of these unusual acyl constituents is not yet known.

A second point is that under conditions of essential fatty acid deficiency (EFAD), endogenously synthesized oleic acid (18:1ω9) can be elongated and desaturated, mainly to 20:3ω9 and 22:3ω9. Thus, membranes from animals with EFAD contain PUFAs, although they lack the "essential" double bonds in either the ω6 or ω3 positions.

Susceptibility of membrane fatty acids to dietary modification appears to be organ-specific, with the heart, liver and testes most sensitive and the brain and retina most resistant to dietary change.[24] This may be related in part to preferential uptake of different fatty acids in different body organs/cell types[33] and/or to variability in their abilities to metabolize the precursor essential fatty acids.[33] The high degree of resistance of neural membranes to changes in PUFAs had posed considerable difficulty in addressing questions of their roles in membrane function until recently, when it was demonstrated that the PUFA composition of transformed cells of neural origin could be modified dramatically by additions to or deletions from the culture medium.[34,35] These cells have many of the properties of normal neural cells, including the presence of cell-surface receptors that are functionally coupled to intracellular effectors (e.g., adenylate cyclase), and, as such, have proven to be excellent models for studies of the relationship between membrane PUFAs and protein function.

Mechanisms of fatty acid modulation of membrane protein function

There are at least three distinct mechanisms whereby fatty acids can affect the functioning of integral and intrinsic proteins. These include (1) direct effects of the fatty acids on protein structure and/or mobility

(see below); (2) modification of protein function through posttranslational, covalent binding of fatty acids, or complex lipids; and (3) the potential of the fatty acids (e.g., arachidonic acid) to be substrates for synthesis of bioactive metabolites, including the eicosanoids and lipid peroxides. This review will concern specifically those proteins whose functions are modulated by direct interaction with the fatty acid environment.

The fatty acids esterified to complex lipids constitute the hydrophobic core of the membrane that provides anchorage for the many integral and intrinsic proteins that function as enzymes, receptor complexes, transporters, and ion channels.[24,25] One means by which they could influence the functioning of a protein is by affecting its conformation *per se* (e.g., by becoming imbedded in folds in the hydrophobic regions of the protein), and/or by imposing constraints on conformational changes. Each of the proteins embedded within the membrane is surrounded by a single-layered lipid shell, referred to as the "boundary" lipid or lipid annulus (*Figure 1*), the hydrophobic portions of which serve to solubilize the protein.[36,37] It is generally believed that the closely associated lipids are highly ordered structures;[38] however, the acyl chains could readily exchange with those in the bulk phase of the membrane via a deacylation/reacylation process. Thus, it is reasonable to assume that major changes in bulk composition could be reflected in changes in the region close to the protein, which in turn may influence its shape and, therefore, its ability to function.

Membrane fatty acids can also directly affect the dynamic properties of a protein (or protein complex) through their influences on the bulk physical characteristics of the hydrophobic core of the bilayer. In general, the bilayer tends to exist at the transition point between a fluid ("liquid crystalline") and solid-like ("gel") state; the phospholipid fatty acyl chains are one of the main chemical determinants of the balance between the two (the others being the phospholipid head group composition and the cholesterol content). *cis*-Polyunsaturated fatty acids of the ω6 and ω3 series tend to increase the "fluidity" of the membrane, a

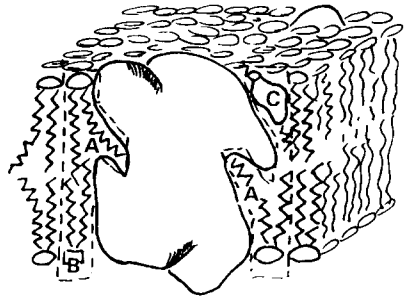

Figure 1 "Boundary" lipid (lipid annulus) surrounding the membrane protein influences its conformation and ability to change conformation. (A) Fatty acids esterified to phospholipids intercalate into hydrophobic regions of the protein. (B) The very long acyl chains of sphingolipids may extend into the opposing leaflet of the bilayer and/or may increase the thickness of the membrane. (C) Cholesterol "packs" membrane fatty acids and confers rigidity to the membrane proteins.

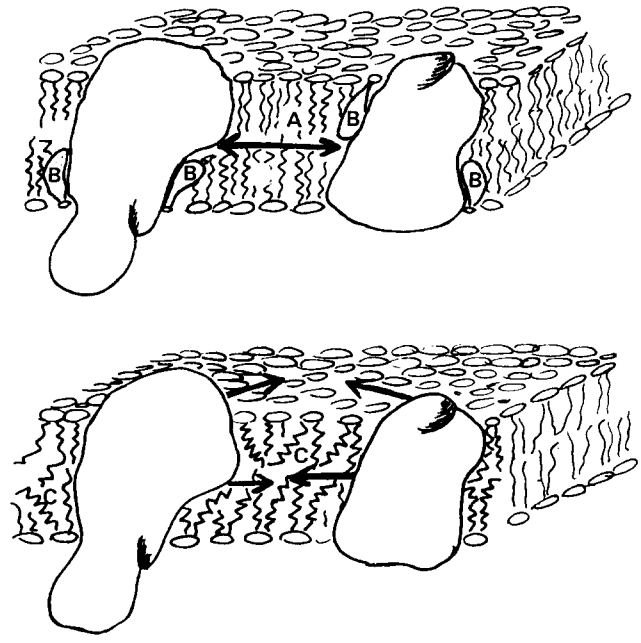

Figure 2 Membranes that contain large amounts of straight-chained saturated fatty acids (A) and/or cholesterol (B) are less "fluid" than those that contain high proportions of *cis*-PUFAs of the ω6 and ω3 series (C). The "fluid" environment enhances protein mobility, and will affect functions that involve protein aggregation, such as receptor-effector interactions.

parameter that refers to the rate of motion of the acyl chain and to its orientation, or "packing," within the membrane. In the past, it was frequently assumed that the greater the number of double bonds in a fatty acid, the greater the membrane "fluidity"; however, we now appreciate that the relationship is far from a simple one.[24] Polyunsaturates also influence the ability of the membrane phospholipids to deviate from the bilayer configuration and form micellar or cone-like structures, both of which also influence membrane physical properties.[39,40] Unsaturated fatty acids also appear to play a role in determining the asymmetric distribution of sterols within the bilayer, and, in doing so, have the potential to alter membrane order in a highly localized manner.[41] The influences that fatty acids exert on the fluidity and/or order of a membrane have widespread implications for the functioning of those proteins whose actions depend on mobility within the plane of the membrane (*Figure 2*). These include such events as receptor aggregation, interactions between receptors, nucleotide regulatory proteins and their intracellular effectors, and receptor desensitization or down-regulation.[42]

Other, less well-documented mechanisms whereby fatty acids may influence protein function include their possible effects on membrane thickness[33,43] and on protein synthesis.[33,44]

Fatty acid modulation of membrane-bound enzyme activities

This review will focus on three plasma membrane-bound enzymes that have been demonstrated to be

particularly sensitive to their fatty acid environments. These are adenylate cyclase (ATP pyrophosphate-lyase [cyclizing], EC 4.6.1.1), 5′-nucleotidase (EC 3.1.3.5), and Na^+/K^+ ATPase (EC 3.6.1.37).

Adenylate cyclase (ACase) is one of the major enzymes involved in transmembrane signaling and, as such, is a key regulator of cell function. The importance of the membrane lipid environment in regulating its activity has been appreciated for some time (for review, see Houslay[45]). One system that has been extensively studied is that of the β-adrenergic/ACase system of cardiac membranes. Alam et al. recently demonstrated that basal, fluoride-, forskolin-, and isoproterenol-stimulated ACase activities were significantly reduced in cardiac membranes from rats fed diets either deficient in essential fatty acids (i.e., containing 7% coconut oil)[46] or containing *trans*-octadecenoic acid (t-18:1ω9) as their major component.[47] Control diets contained equivalent proportions of corn oil. Neither treatment affected the double bond index (DBI) of the membranes, but both were associated with an increase in the amount of ω9 PUFA. In both studies, the decreased enzyme activity was accompanied by a reduction in the number of β-adrenergic receptors, as judged by a reduction in the binding of [^{3}H]dihydroalprenalol (DHA) (*Table 1*). Interestingly, in the EFAD study,[46] dietary repletion restored normal membrane fatty acid profiles without fully reversing the attenuated enzyme activities. Subsequently, the same investigators found that both basal and stimulated ACase activities were significantly increased in rats fed diets that contained 10% menhaden oil, which is particularly rich in eicosapentaenoic (C20:5ω3) and docosahexaenoic (C22:6ω3) acids.[53] Based on observations that there were increases in the B_{max} for binding of [^{3}H]forskolin but not for [^{3}H]DHA, they concluded that the basis of the increased enzyme activity was a selective PUFA-dependent increase in the synthesis of the ACase catalytic unit. The fact that forskolin-stimulated ACase was also elevated in these membranes supported this conclusion. In preliminary studies, Hamm and Shei[52] reported that isoproterenol-stimulated ACase was also elevated in cardiac membranes from rats fed diets rich in ω6 PUFA, and that this increase was also associated with increases in β-adrenergic ligand binding. In comparison, McMurchie et al. found that in cardiac membranes from both rats[49] and marmoset monkeys,[50] the highest isoproterenol-stimulated ACase was associated with a decrease in total ω6 PUFA and an increase in the relative proportion of ω3 polyenes, particularly docosahexaenoate (22:6ω3). Interestingly, the high ω3 content was observed in animals that were fed diets high in saturated fat (sheep kidney fat), which suggests that under conditions of relative dietary deprivation, membrane systems will select and retain particular PUFA species. Although the results of the studies cited above suggest that increasing cardiac PUFA content is in some way associated with increased responsiveness of ACase to adrenergic stimulation, not all investigators have come to this conclusion. Several groups have reported that increasing dietary linoleic acid (e.g., diets containing sunflower oil) decreased β-adrenergic receptor-induced ACase activity in rat cardiac ventricle[54] and atrial homogenates[51] and in isolated papillary muscle.[55] Laustiola et al.[56] found that atria from rats fed diets supplemented with cod liver oil (i.e., a ω3 PUFA-enriched diet) had low basal and isoproterenol-stimulated accumulation of cAMP, which argues against a fatty acid-specific attenuation effect.

Contradictory data clearly emphasize the need for

Table 1 Effects of dietary fatty acids on adrenergic receptor binding in the heart

Membrane source	Dietary manipulation	Effect on DBI	Effect on ω6/ω3	Ligand	Effect on B_{max}	Effect on K_d	Reference
Rat heart	EFAD	→	NA	[^{3}H]DHA	↓	↓	46
Rat heart	↑ *trans* acids	→	NA	[^{3}H]DHA	→	→	47
Rat heart	↑ saturated fat	NA	NA	[^{3}H]DHA	↑	↑	48
Rat heart	↑ saturated fat	→	↓	[^{125}I]ICP	→	→	49
Marmoset heart	↑ saturated fat	→	↓	[^{125}I]ICP	↓	→	50
Rat heart	↑ linoleic acid	NA	NA	[^{3}H]DHA	→	↓	48
Rat atrial homogenates	↑ linoleic acid	→	NA	[^{3}H]DHA	↓	↓	51
Rat heart	↑ ω6 PUFA	NA	NA	[^{3}H]DHA	↑	↑	52
Rat heart	↑ linoleic acid	→	↑	[^{125}I]ICP	→	→	49
Marmoset heart	↑ linoleic acid	→	↑	[^{125}I]ICP	→	→	50
Rat heart	↑ menhedan oil (ω3 PUFA)	↑	↓	[^{3}H]DHA	→	↑	53

Abbreviations: NA, information not available; ICP, iodocyanopindolol.

caution in comparing results from different studies. Many factors must be taken into account, such as the composition of the individual diets (including reference diets) and the membrane changes that they elicit, the latter of which reflect specificities in the ability of different species, tissues, and/or cell types to select, metabolize, and incorporate different fatty acids.

Adenylate cyclase activity in liver plasma membranes has also been demonstrated to be sensitive to membrane fatty acid content. Recently, Lee and Hamm[57] showed that there were diet-dependent increases in fluoride-, forskolin-, and glucagon-stimulated ACase activities that followed the order fish oil > corn oil > butter. There were no differences in basal ACase activities between the three groups, and the numbers and affinities of glucagon receptors were likewise unaffected. The investigators concluded that the fatty acid-dependent effects occurred distal to receptor occupation, perhaps at the stage of coupling of the G protein and catalytic units. The results of this study are supported by earlier reports that glucagon-stimulated ACase was reduced in membranes from rats with EFAD[58] and was increased in membranes with increased linoleic acid content.[59] In contrast, Clandinin and coworkers[60-62] found that there was a negative correlation between glucagon-stimulated ACase and increasing liver membrane content of ω6 and ω3 PUFAs. Dietary fatty acid content was manipulated in several ways, such as by replacement of linoleic with oleic acid or by combining different natural oils, including linseed and olive oils. Lee and Hamm[57] attributed the discrepancies between the different studies to variations in both the amounts and types of fatty acids in the dietary regimens. One must also consider the possibility that the levels of endogenous circulating hormones could be affected by dietary status, and that apparently contradictory effects may be due to adaptive changes, such as receptor down-regulation.

Relatively few studies have examined the effects of fatty acids on adenylate cyclase activity in neural membranes due to their resistance to dietary modification *in vivo*.[5] However, we and others have recently addressed this question in studies in which the PUFA content of transformed cells of neural origin has been increased dramatically by adding PUFAs (e.g., arachidonic acid, docosahexaenoic acid) or their precursors (e.g., linoleic acid, linolenic acid) to the culture medium. We recently demonstrated that both basal and prostaglandin E_1-stimulated adenylate cyclase activities were increased significantly in N1E-115 neuroblastoma cells that had been cultured in the presence of linoleic acid.[35,63] These findings contradicted the earlier reports of McGee and Kenimer,[64] who found that neither activity was affected in membranes prepared from similarly treated NG108-15 neuroblastoma × glioma hybrid cells. In studies to resolve this discrepancy, we found that in both types of cells, enhancement required an intact cell preparation. We tentatively concluded that the PUFA effect was lost as a result of homogenization-induced activation of phospholipase(s) and disruption of subtle but critical lipid-protein interactions. However, we recently demonstrated that the PUFA-stimulated "basal" cyclase activity is blocked (>80%) by treatment with adenosine deaminase or 8-phenyltheophylline, suggesting that the enhanced "basal" activity is almost completely due to PUFA-dependent increases in the production of extracellular adenosine, which then activates ACase-stimulating receptors.[65] This argues against significant modulation of basal activity of the catalytic unit *per se* by boundary PUFAs in this cell line. Further studies indicate that adenosine receptor-ACase coupling was altered by PUFA enrichment of the neuroblastoma cell membranes, since the maximum amount of cAMP formed in response to N'-ethylcarboxyamide adenosine was significantly higher in the supplemented cells, and that the amount of agonist required for half maximal stimulation was significantly reduced.[66] Recent data have suggested that these effects may be due, at least in part, to PUFA-dependent increases in the number of cell-surface adenosine receptors (unpublished observations).

For many years, it was assumed that increases in adenylate cyclase activities in PUFA-enriched membranes were due primarily to fatty acid-dependent increases in membrane fluidity.[45] However, there are data indicating that in some tissues and/or with some types of receptors, enrichment has either no effect or decreases receptor-ACase interactions. There are several reports of inhibitory effects in cardiac membranes (discussed above). In 1986, Alam and Alam[44] reported that basal ACase was unaffected by EFAD in plasma membranes from rat submandibular salivary glands, but that fluoride-stimulated activity was enhanced by approximately 35% in the deficient membranes. These membranes had a reduced membrane fluidity, as judged by increased fluorescence polarization. In keeping with these findings, Sebokova et al.[67] recently demonstrated that fluoride-stimulated ACase was significantly reduced in testicular plasma membranes from rats fed diets enriched in either linoleic or linolenic acids, and that leutinizing hormone-stimulated activity was reduced in the order linolenate > linoleate. Receptor binding studies suggested that the latter was due to PUFA-dependent decreases in hormone receptor density and not to alterations in agonist-receptor affinities. In our own studies,[63] we demonstrated that enriching neuroblastoma cell membranes in ω6 PUFA by supplementing the culture medium with linoleate did not affect the ability of opiate peptides to inhibit ACase, but did result in a reduced efficiency of coupling between opiate receptors and ACase, as judged by an increase in the amount of opiate required to maximally inhibit ACase.

Overall, the evidence suggests that ACase is affected in a specific manner by membrane fatty acid composition, with increases in PUFAs leading to stimulated activity in some membranes and with some receptors, and reduced activity in others.

5'-Nucleotidase is a membrane-bound ectoenzyme

that is considered a "marker" enzyme for plasma membranes. It catalyzes the dephosphorylation of nucleoside monophosphates, such as adenosine 5'-monophosphate (5'-AMP) to adenosine, and, as such, plays a potentially important role in the modulation of neural, cardiovascular, and immune functions. Evidence suggests that it exists as a lipoprotein complex in a sphingomyelin-rich environment,[68] anchored into the membrane via a covalent linkage to phosphatidylinositol.[69] With relatively few exceptions,[70,71] diet-induced reductions in membrane PUFAs appear to be associated with decreased 5'-nucleotidase activity. In early studies, investigators found that EFAD-dependent decreases in ω6 PUFA led to 25% to 50% reductions in the specific activities of 5'-nucleotidase in liver[72] and cardiac[58] membranes. Haeffner et al.[73] reported a similar decrease (30% to 50%) in membranes from ascites tumor cells cultured for 1 day in a lipid-free medium. Consistent with these findings were the observations that liver[68] and cardiac[74] membranes from rats fed diets enriched in ω6 PUFA (i.e., sunflower seed oil) were increased relative to those from animals fed hydrogenated sunflower oil or coconut oil. Momchilova et al.[68] indicated that the high PUFA diet was associated with both an increased DBI and an increase in the ratio of sphingomyelin/phosphatidylcholine. Zuniga et al.[33] recently examined the effects of dietary menhaden oil on 5'-nucleotidase activity in rat liver plasma membranes. After 3 weeks, the plasma membranes were enriched in ω3 PUFA and had specific enzyme activities that were 1.6 to 1.8 times higher than those in membranes from rats fed corn oil (high ω6 PUFA) or coconut oil (high saturated fat), even though the unsaturation indices were similar for all three groups. The ω3 PUFA-dependent elevation in enzyme activity reflected an increase in its V_{max}, with no change in its apparent affinity for substrate. Activities in membranes from the corn and coconut oil groups were equivalent, although there was a marked difference between their ratios of ω6 to ω3 PUFA. The profiles of temperature dependence of the energies of activation (i.e., Arrhenius plots) for the fish oil-fed versus coconut oil-fed rats also could not account for the differences in enzyme activities, which led the investigators to propose that the ω3 PUFA had a direct, fatty acid-specific effect on the conformation of the enzyme. This proposal had been made earlier by Bernsohn and Spitz[75] when they found that the significant (>60%) reductions in 5'-nucleotidase activity in brain membranes from rats fed a fat-free diet for 4 months could be reversed by linolenic but not linoleic acid feeding. Consistent with this, Bourre et al.[7] recently reported that diet-induced decreases in ω3 PUFA (e.g., diets containing sunflower, peanut, soya, or rapeseed oil) resulted in a 20% reduction in enzyme activity in rat neural membranes. However, Hannah and Campagnoni[70] found that 5'-nucleotidase was not significantly altered (and tended to be elevated) in brains of mice that had been maintained on an EFAD diet during the last week of gestation and for 16 weeks postpartum. Whether this discrepancy reflects a species-specific or an age-dependent effect remains to be determined.

Na^+/K^+ ATPase, or the sodium pump, is a multi-unit, multi-function protein system located in the plasma membrane of most cells. It contains intracellular sites for ATP hydrolysis and phosphorylation, as well as intracellular and extracellular sites for ion (Na^+,K^+) binding and translocation. It is also the only ion pump that contains a receptor.[76] Approximately half of the molecular mass of the complex is lipid (250 to 300 mol phospholipid/1 mol enzyme,[77] and its specific requirements for negatively charged phospholipid for integrity and activity have been well-documented.[77-79] In contrast to the situation with 5'-nucleotidase, increases in membrane PUFAs reduce rather than enhance the activity of Na^+/K^+ ATPase. Several groups of investigators have reported small but significant increases in brain ATPase activities in mice maintained for long periods on EFAD diets.[70,80] Na^+/K^+ ATPase activity was also increased in liver membranes from rats fed EFAD diets.[58] The changes in the liver involved increases in the values for V and Km for the substrate; however, there was no change in the Arrhenius plot. This apparent negative correlation between enzyme activity and membrane PUFA content had been reported earlier by Bloj et al.,[81] who found that allosteric interactions (as judged by decreasing Hill coefficients) decreased as the unsaturation index of the membranes increased. Increases in enzyme activity have also been reported in EFAD membranes from the kidney[71,82] and salivary glands.[71] Similar effects were seen in animals fed diets high in saturated fats (i.e., butter), which led Alam and Alam[71] to suggest that modifications in Na^+/K^+ ATPase were directly related to EFAD- or saturated fat-dependent decreases in membrane fluidity. This hypothesis was supported by the data of Abeywardena et al.,[83] who found that Na^+/K^+ ATPase activities were the same in cardiac sarcolemmal membranes that had similar fluidity properties regardless of whether they had a higher content of ω6 PUFA (sunflower seed oil diet) or ω3 PUFA (sheep kidney fat diet). Alam et al.[47] recently challenged this concept when they reported that ATPase activity was reduced significantly in cardiac membranes from rats fed diets containing high proportions of *trans*-octadecenoic acid (t-18:1), even though the "fluidity" of the membranes was unchanged relative to that from control animals.

Although the specific means by which membrane PUFAs modulate Na^+/K^+ ATPase activity are not well-understood, several mechanisms in addition to their effects on environmental fluidity have been suggested. These include fatty acid-dependent effects on (1) the conformational (e.g., allosteric) properties of the protein complex, (2) the amounts of enzyme,[71,82] and (3) the thickness of the phospholipid bilayer.[43] One of the most interesting aspects of the observation that Na^+/K^+ ATPase activity in many diverse membrane systems is increased with decreases in membrane PUFAs is that this may reflect the need of the enzyme complex to adapt to structural alterations imposed by

dietary stress to retain its essential role in maintaining ion homeostasis.

Modulation of receptor function

The transmission of information across biologic membranes involves at least three membrane-bound proteins, namely, the recognition site or "receptor," a nucleotide regulatory protein ("G protein"), and an effector protein such as an enzyme or an ion channel. As discussed above, fatty acids are potentially important modulators of receptor function by virtue of their effects on protein conformation as well as on the mobility of the components within the plane of the membrane. For a review of earlier studies of the role of lipid environment in receptor function, see Loh and Law.[84] Many of the recent dietary studies have focused on the effects of fatty acids on receptor density (B_{max}) or the strength of the interaction (i.e., affinity, or K_d) between the ligand and the receptor (*Table 1*), in addition to their modulation of the "effector" proteins (e.g., ACase) described above.

Opiate receptors

The results of early studies of the effects of perturbation of membrane lipids suggested that opiate receptors were sensitive to their lipid environment.[84] In 1982, McGee and Kenimer[64] reported that increasing the ω6 PUFA content of neuroblastoma × glioma cells by supplementing them with either linoleic (18:2ω6) or arachidonic (20:4ω6) acids resulted in a decrease in the capacity of the membranes to bind radiolabeled opiate ligands without altering their binding affinities. Ho and Cox[86] obtained similar results with this cell line, regardless of whether it was supplemented with linoleic or linolenic (18:3ω3) acids. Our studies with intact N1E-115 neuroblastoma cells indicate that supplementation with linoleate does not affect opiate receptor density (measured by binding of [^{3}H]DADLE), but reduces the affinity of ligand binding.[63] This effect seems to be receptor-specific in this cell line, since preliminary studies have indicated that under the same supplementation conditions, the density of adenosine receptors increases, while the affinity of ligand binding remains unchanged (unpublished observations).

Adrenergic receptors

As discussed above (see the adenylate cyclase section), there is strong evidence that adrenergic receptor function, particularly in the heart, is strongly influenced by the membrane lipid environment. Several groups of investigators have examined the effects of diet on adrenergic ligand binding.[46,47,49,50,52,53,87] As shown in *Table 1*, there are many apparent contradictions in the data; however, as discussed above, the inconsistencies could reflect a host of different factors, including the amounts and specific types of fatty acids in each diet, the duration of the diet, the species being examined, and the conditions of the binding assay. Individual studies are most beneficial when they use more than one approach to examine the effects of diet on receptor function. For example, Wince and colleagues[51,88] demonstrated not only that β-receptor density and ligand affinity decreased in atrial tissue from rats fed diets high in ω6 PUFA (sunflower seed oil), but that these reductions correlated with attenuated adrenergic stimulation of ACase (see above) and contractile force. In subsequent studies designed to determine the molecular mechanism(s) of fatty acid modulation, the investigators determined that the ω6 PUFA enrichment resulted in perturbation of the ACase-cAMP pathway at a point(s) distal to the catalytic unit, although phosphodiesterase activity was not affected. Fatty acid modulation of receptor-mediated events may not always involve functional changes in the interaction between ligand and receptor. For example, Loesberg et al.[48] reported that isoprenaline-induced relaxation of guinea pig tracheal spirals was maximal when the semisynthetic diets contained moderate amounts (11.3 energy %) of linoleic acid, and were reduced when its proportion was either decreased or increased; however, none of these changes altered β-receptor density or binding affinity.

From a different perspective, at least two groups of investigators have shown that stimulation of adrenergic receptors can induce changes in membrane fatty acid composition. Benediktsdottir and Gudbjarnason[89] recently demonstrated that chronic exposure of animals to adrenergic agonists results in dramatic increases in the cardiac membrane content of docosahexaenoic acid. In studies using rat brains, Barkai and Murthy[90] found that noradrenaline (as well as serotonin) stimulated the selective incorporation of arachidonate into phosphatidylinositol, an effect they suggested was due to neurotransmitter-dependent activation of lyso-phosphatidylinositol arachidonyl transferase. Neither phenomenon has been extensively documented; however, both reinforce the concept of a dynamic interaction between adrenergic receptors and their associated fatty acids.

Insulin receptors

Observations that diabetes mellitus is rare in Eskimos from Alaska and Greenland led Lardinois to recently propose that this is at least partly related to the high ω3 PUFA content in the diets of these populations.[91] He suggested that increases in the membrane content of ω3 PUFA enhanced interactions between insulin and its receptor, and optimized glucose-stimulated insulin secretion, the latter of which involved ω3-dependent blockade of prostaglandin formation. However, reports from others have indicated that diets high in ω6 PUFA can also potentiate insulin receptor function. Increasing membrane ω6 PUFA by feeding animals safflower oil (versus hydrogenated beef tallow or palm oil) has been shown to increase the amount of insulin bound to adipocyte plasma membranes,[92,93] due to an

increase in the number of low-affinity binding sites, and to increase the maximum amount of glucose taken up in response to insulin.[93] Consistent with these findings, adipocytes from rats fed diets high in saturated fat (mainly lard) bound less insulin and had a reduced insulin-dependent uptake of 2-deoxyglucose than did control (corn oil-fed) animals.[94] Of possible therapeutic value was the observation that feeding diabetic rats diets high in linoleate was sufficient to elevate the amount of insulin bound to levels that were equivalent to those in control animals.[92] The effects of ω6 PUFA at the membrane level may be tissue specific; this is supported by the finding of Watarai et al.[94] that binding of insulin to receptors solubilized from liver was not affected by the high-fat diet, although insulin-stimulated phosphorylation of the β-subunit of the receptor was significantly reduced in these preparations. In supplementation studies with an insulin-sensitive hepatoma cell line, Bruneau et al.[95] found that the number of total binding sites for insulin were decreased in cells cultured with linoleic acid, although the number of high-affinity sites was increased. These investigators also found that the time course of insulin-induced receptor down-regulation was accelerated by ω6 PUFA enrichment, as was maximal insulin-dependent stimulation of glycogen synthesis.[96] Pelikanova et al.[97] recently reported that there were strong negative correlations between diet-induced increases in the linoleate and arachidonate contents of erythrocyte membranes from healthy men and the amounts of insulin bound to the erythrocytes.

With the growing interest in the effects of the lipid environment on membrane function, investigators are beginning to examine the effects of dietary fatty acids on the physiologic responses to receptor activation. Data from a variety of studies indicate that they can affect many membrane responses, including peripheral vasoconstriction,[98] cardiac inotropic responsiveness,[86,87,99,100] coronary microvessel relaxation,[101-103] bronchoconstriction,[104,105] and neurotransmitter release.[96,106,107] In most of these studies, the molecular mechanisms of the observed changes have not been examined.

Other membrane functions modulated by fatty acids

The list of protein functions that are influenced by membrane fatty-acid composition continues to grow. One particularly exciting area of research is that concerning the effects of ω3 PUFA on retinal photoreceptor function. Connor and Neuringer[4] recently reported that depriving very young rhesus monkeys of ω3 fatty acids led to quantitative replacement of these acids by ω6 polyenes, and that this was associated with abnormalities in visual system function. Electroretinogram recordings indicated that the rod and cone components of ω3-deficient animals exhibited both delayed and reduced recovery of the dark-adapted response after exposure to light flashes. Interestingly, the retinal fatty acid profiles, but not the impairments in visual function, were readily returned to normal if the animals' diets were repleted with ω3 PUFA, a finding that supports the view that there is a narrow window during the early stages of development when the presence of this class of PUFAs is essential for normal function to occur.

Other protein-dependent processes that have been demonstrated to be affected by membrane fatty acid composition are those that involve transport of electrolytes, sugars, amino acids, and other small molecules (for reviews, see refs 20, 25, and 27). The numerous studies, which have most frequently involved supplementation of cultured neural cells, have not yielded any "rule of thumb" regarding specific effects; rather, the responses to PUFA enrichment appear to depend on the molecule being transported, the fatty acid(s), and the cell line. Nevertheless, the results of these studies suggest that the fatty acid environment modulates transport in a manner that favors maintenance of the intracellular or extracellular concentrations of metabolites within those limits that are required for cell viability.[25] The molecular bases proposed for the regulatory effects include the effects of the acyl chains on membrane thickness and order.[27] In a recent study, Schneeberger et al.[108] concluded that the formation of tight junctions could be regulated by membrane PUFAs when they demonstrated that establishment of functional junctions took longer in MDCK cells that were supplemented with γ-linolenic acid (18:3ω6) than in cells that were cultured with α-linolenic (18:3ω3) or oleic acids.

Increases in membrane PUFA content have been reported to alter the electrical properties of cultured cells of neural origin. Love et al.[109] demonstrated that culturing NG108-15 hybrid cells in the presence of linoleic, linolenic, or arachidonic acids resulted in decreases in the rates of rise and amplitudes of Na^+ action potentials. Neither resting membrane potentials nor Ca^{++} action potentials were altered in the PUFA-enriched cells. Grynberg et al.[110] found that there was no difference between the electrical or mechanical activities of rat ventricular myocytes cultured with linoleic or linolenic acids under normoxic conditions; however, there were fatty acid-specific effects on the cellular responses to hypoxic insult. The action potential depression under hypoxia was less severe in myocytes that were enriched in ω6 PUFA, whereas there was better electrophysiologic recovery on reoxygenation in cells supplemented with linolenic acid (i.e., ω3 PUFA enriched). Our own studies[111] demonstrate that the final phases of repolarization in ventricular myocytes freshly isolated from rats fed cod liver oil-rich diets were consistently more positive than those in myocytes of control animals. These differences may have been related to changes in membrane conductance in the cod liver oil-fed rats, a suggestion that is supported by observations that $^{45}Ca^{++}$ uptake was significantly higher in their myocytes. Ca^{++} efflux from these cells was not affected by dietary status.

Conclusion

From the information presented in this review, it is increasingly evident that the many different fatty acids that constitute the hydrophobic core of biologic membranes have the potential to directly modulate the functioning of a host of membrane proteins in a manner that reflects specificity with respect to protein, membrane, fatty acid, and, perhaps, species. Earlier, the modulatory functions of the fatty acids were mainly attributed to their influences on the bulk fluidity properties of the membrane, and the consequent effects on the ability of proteins, such as those forming receptor complexes, to migrate within the plane of the membrane. We now appreciate that other factors could be involved, including the potential abilities of fatty acids to stabilize optimal protein conformation, to determine the thickness of the membrane, and/or to regulate protein synthesis. At this juncture, it is not possible to identify specific molecular mechanisms in each of the examples we have discussed, largely because much of the information is still empiric and fragmentary in nature. In only a few cases have there been sufficient independent, consistent observations for us even to begin to speculate on the mechanisms involved. What is clear, however, is that only with elucidation of these mechanisms will we understand the basis for the essentiality of particular dietary fats, and thereby be able to use them to their fullest potential.

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