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# Dietary Lipids and the Regulation of Membrane Fluidity and Function

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## INTRODUCTION

With the understanding and virtual elimination of the deficiency diseases, developed western societies now face a variety of chronic diseases as their primary health problem. For the "diseases of affluence," such as coronary heart disease, cancer, stroke, hypertension, and diabetes mellitus, there is increasing evidence that nutritional factors are major etiological determinants, with the amount and type of dietary fat being particularly important (Trowell, 1981). Dietary intake of saturated (animal) and unsaturated (vegetable and fish) fat, as well as cholesterol and fiber, controls, in part, the plasma cholesterol level, with elevated levels being implicated in atherosclerosis and coronary heart disease (Oliver, 1982). It is also becoming increasingly apparent that dietary lipids may exert an additional and profound influence on cell function because of their effects on biological membranes (Kummerow, 1985). In that some of the basic mechanisms associated with the "diseases of affluence" are often characterized by altered cell function at the membrane level (Nicolson, 1976b; Shinitzky, 1984a; Holman, 1986), the effect of dietary lipids on membrane properties becomes an important issue in human health and nutrition.

## SCOPE OF THE REVIEW

This review highlights some of the effects dietary lipids exert on membrane lipid composition, membrane fluidity, and membrane functional activity. Membrane lipids and membrane architecture are reviewed briefly to emphasize the inherent complexity and compositional diversity of biological membranes, which hinder our full understanding of the nature of the membrane changes induced by dietary lipids.

This review does not cover the effects of dietary lipids on the production of eicosanoids or other lipid messengers, or effects on the immune response, both of which have been recently reviewed (Hansen, 1983; Johnston, 1985). This review deals primarily with dietary lipid effects on mammalian membranes. Studies using other nonmammalian vertebrates, including birds, are relatively few in number and do not add significantly to the results obtained using mammals. A recent review of membrane lipid modification of cultured animal cells can be found elsewhere (Spector and Yorek, 1985), and this subject is not covered in this review.

## STRATEGIES, RATIONALE, AND EXPERIMENTAL APPROACHES

Two lines of evidence support the concept that dietary lipids influence cell function by

their effect on biological membranes. First, the lipid composition of membranes is not static but can be altered by manipulation of the dietary lipid intake in experimental animals (Wahle, 1983; Stubbs and Smith, 1984; Clandinin et al., 1985) and man (Dyerberg, 1986), by selective supplementation of the medium of cultured cells (Spector and Yorek, 1985), and by in vitro manipulation of the lipid composition of isolated cells and membranes (Borochov and Shinitzky, 1976; Breton et al., 1983; O'Conner et al., 1984; Lochner et al., 1984). Second, it is established that many membrane proteins, involved in roles critical to cell function, become so only when associated with a suitable membrane lipid environment (Sandermann, 1978; Quinn, 1981) and that an "optimal" lipid environment within a membrane is the normal requirement for full expression of functional activity (Shinitzky, 1984a). Further supporting the concept of lipid modulation of membrane function is our increasing understanding of the structure and dynamics of biological membranes and the nature and implications of lipid–lipid and lipid–protein interactions in the membrane. Lipid–lipid interactions determine the physical properties of the membrane and depend on the composition of the membrane lipids (Quinn, 1981). Lipid–protein interactions allow the activity of membrane proteins to be modulated by changes in the physical properties of the membrane lipids (Sandermann, 1978; Loh and Law, 1980; Benga and Holmes, 1984).

The evidence cited above sets the experimental strategy and rationale for studying dietary lipids, membrane physical properties, and the regulation of membrane function. The experimental approach involves modifying the lipid composition of nutritionally complete diets and feeding experimental animals for a period of time sufficient to induce and maintain a change in the lipid composition of the membrane of interest. Following analysis of the membrane lipid composition, some biophysical technique is often used to determine whether membrane physicochemical properties (e.g., membrane fluidity) have been altered. Measurements are also made on various functional aspects associated with the membrane such as an enzyme activity or receptor number and/or ligand-binding affinity. By following this protocol, the investigator would hope to determine the effects of a particular dietary lipid regime on membrane composition and function and rationalize the observed changes as resulting from alterations in membrane physical properties.

Although overall, the above approach is justifiable, the experimental outcome often depends on the particular dietary lipid supplement, the choice of experimental animal, the membrane function under study, and the biophysical technique employed to determine membrane physical properties. Predicting the effects of dietary lipids at the membrane level is difficult for a number of reasons. The lipid composition of the membrane may not respond to the influence of dietary lipid in the manner anticipated because of homeostatic mechanisms designed to minimize perturbation to membrane functions (Sinensky, 1974). Also, membrane physical properties may not correspond to those expected from the lipid composition. The determination of physical properties, such as membrane fluidity, may be subject to interpretational and technical difficulties (Keith et al., 1973; Schreier et al., 1978). Finally, for similar changes in membrane lipid composition, the activities of membrane-associated functions may be affected in opposite ways, or, indeed, not at all, depending on their particular association with the membrane lipids (Johannsson et al., 1981; Houslay, 1985).

## MEMBRANES AND MEMBRANE FLUIDITY

### Membrane Architecture

Although membranes are dynamic structures, they still maintain basic architectural features that govern their function and behavior. Whereas dietary modification of membrane lipid composition would not be expected to alter membrane architecture significantly, it may influence some of the more

dynamic aspects of the membrane, resulting in modulation of membrane function. The overall architecture of biological membranes, i.e., the supermolecular arrangement of lipids and proteins, is well understood and is based on established physical principles. The existence and maintenance of a lipid bilayer structure and the preference that integral membrane proteins display for location within such an environment are the result of free energy considerations in relation to both hydrophobic and hydrophilic interactions (Singer and Nicolson, 1972). The amphipathic nature of the membrane lipids ensures that for the most part a stable bilayer (lamellar phase) is maintained, although the hexagonal ($H_{II}$) phase conformation may be the preferred orientation of some phospholipids under certain conditions (Cullis and de Kruijff, 1979). For integral membrane proteins, some property of their amino acid residues, probably related to the tertiary structure and the position of hydrophobic and hydrophilic residues relative to the membrane lipids rather than the overall proportion of each class of amino acid residue, dictates the optimal position a protein will assume in the lipid bilayer (Singer and Nicolson, 1972). Positional coincidence of charges on the amino acids and the head groups of membrane phospholipids, on the one hand, and the proximity of the hydrophobic amino acid residues to the hydrocarbon acyl fatty acid interior of the bilayer, on the other, would be expected to stabilize proteins in the bilayer by maximizing electrostatic and hydrophobic interactions. These interactions could influence the vertical position of a protein within the bilayer or the conformation of an active or regulatory site of a membrane enzyme (Shinitzky, 1984a).

Implicit in studies in which a modification of membrane function is achieved by dietary lipid is the recognition that membrane proteins do not undergo any major change per se. Rather, alterations in the membrane physicochemical properties influence membrane enzyme conformation, the rate of lateral or rotational diffusion of proteins in the membrane, or the vertical positioning of a protein in the lipid bilayer. The need to maintain maximum structural stability of the membrane by the weak interactive forces between lipids and proteins could be one mechanism whereby membrane proteins undergo conformational or positional changes in response to altered membrane lipid properties. However, it is quite conceivable that, as a result of lipid-induced changes in membrane function, secondary changes directly involving membrane proteins, such as receptor down-regulation, may occur.

The association of membrane proteins with the lipid bilayer is variable. Cytoskeletal interactions restrict some proteins to particular positions in the lateral plane of the membrane (Nicolson, 1976a), whereas other proteins may be anchored in the membrane by a hydrophobic domain, while the bulk of the protein exists in the aqueous phase on either side of the membrane (Shinitzky, 1984a). Other classes of membrane proteins may be located substantially within the lipid bilayer. Repositioning of proteins in the vertical plane of the membrane as a result of altered physical properties of the associated lipids would be expected to influence protein function significantly (Borochov and Shinitzky, 1976). Functional alterations could also occur as a result of changes in the rate of lateral diffusion of proteins, particularly those that are mobile and function as part of an active complex when physical coupling occurs (Helmreich and Elson, 1984). Included in this category are the hormone-stimulated adenylate cyclases (Hanski et al., 1979; Houslay, 1981).

Further degrees of organizational complexity in membranes exist that have relevance to the effect of dietary lipids on membrane functional activity. Lipids and proteins are known to be asymmetrically distributed between the two halves of the membrane, presumably for specialized functional purposes (Op den Kamp, 1979). Distribution of lipids and proteins in the lateral plane of the membrane may also be heterogeneous (Jain and White, 1977). These complexities in membrane architecture

imply that dietary-induced changes in membrane lipid composition do not occur in a uniform manner throughout the membrane. Dietary lipid changes could amplify or suppress membrane activity, depending on the particular lipid environment associated with some functional process. Although effects of dietary lipids on many membrane-associated functional activities have been documented (Wahle, 1983; Stubbs and Smith, 1984; Clandinin et al., 1985), at present the complexity of membrane architecture and the compositional diversity of membrane components hinder a full understanding of the mechanism(s) whereby such effects occur.

## Membrane Lipids

In addition to the architectural complexities, a further degree of difficulty in interpreting the effects of dietary lipids is imposed by the compositional diversity of membrane lipids. It has been estimated that as many as 1,000 different molecular species of lipid exist in the well studied red blood cell membrane (Barenholz, 1984). This diversity is probably increased in more highly functional mammalian membranes. Compositional diversity of membrane (phospho)lipids is due to their unique molecular construction. Superimposed on this is the vast array of spatial and positional variations that membrane phospholipids display within the bilayer. Both of these points limit the extent to which analysis of total phospholipid fatty acid profiles and phospholipid classes can give details of the spatial distribution and dimensional organization of membrane lipids.

For descriptive purposes, membrane lipids can be divided into those molecules containing fatty acyl chains, e.g., phospholipids, and those that in the main do not, e.g., cholesterol. Considerable variation in the structure of phospholipids occurring in the polar head group and the interfacial and hydrophobic regions generates a diversity of structural permutations. For cholesterol, it is of some comfort to the investigator that, generally, structural permutations do not exist in mammalian membranes (Yeagle, 1985). Free fatty acids, triacylglycerols, and cholesterol esters are normally not considered as structural membrane components, although they may play an important secondary role in regulating the supply of fatty acyl groups for incorporation into membranes (Cooper and Strauss, 1984).

Within the phospholipid head group region, where size, shape, polarity, and charge of the head group determine many membrane properties (Boggs, 1986), compositional variation is generated by the presence of a number of distinct bases. Mammalian membrane phospholipids contain predominantly choline and ethanolamine, with serine, inositol, and glycerol occurring to a lesser extent (Quinn, 1981; Stubbs and Smith, 1984; Daum, 1985). In the interfacial region, further variation occurs because of the number and selection of the long-chain fatty acids associated with the particular base. Usually, two fatty acyl chains occur per molecule. However, lysolipids contain only one chain, and sphingomyelin contains a fatty acyl chain in combination with a long-chain amino alcohol base, such as sphingosine. Cardiolipin (diphosphatidlyglycerol) is characterized by four fatty acyl chains per molecule. Diversity of phospholipid structure in the interfacial region is increased by the nature of the bond between the fatty acids and the glycerol backbone. There are diacyl and alkenyl-acyl linkages, the latter giving rise to the plasmalogen form of the phospholipids evident in mammalian cardiac mitochondria (Palmer et al., 1981). With two positions available for the attachment of long-chain fatty acids, many combinations are possible. In most phospholipids, however, the *sn*-1 position is usually occupied by a saturated fatty acyl chain and the *sn*-2 position by a cis unsaturated fatty acyl chain (Stubbs and Smith, 1984). This positional specificity of fatty acyl chains is controlled by the specificity of the acyl transferase enzymes operating during phospholipid synthesis and phospholipid retailoring, as will be discussed below under Membrane Lipid Metabolism and Regulation of Membrane Fluidity.

The greatest compositional diversity of membrane phospholipids occurs within the hydrocarbon region. Mammalian membrane phospholipids are characterized by having fatty acyl chains from 14 to 22 carbons in length, with zero to six double bonds, occurring at different positions along the fatty acyl chain. Although the acyl chain variability could generate many possible combinations, in general the bulk of the acyl chains comprise an even number of carbon atoms and one, two, four, or six methylene-interrupted cis double bonds occurring at characteristic positions. Palmitic 16:0, palmitoleic 16:1 n-7, stearic 18:0, oleic 18:1 n-9, linoleic 18:2 n-6, arachidonic 20:4 n-6, and docosahexaenoic 22:6 n-3 acids predominate in the membrane phospholipids of mammalian cells (Stubbs and Smith, 1984; Holman, 1986).[1] Other variables contributing to fatty acid heterogeneity include the presence of trans and conjugated double bonds, the relative position of the double bond(s), and the presence of different side chains and methyl-branched fatty acid chains.

Cholesterol occurs in the free state in close physical association with the phospholipids (van Dijck et al., 1976; Yeagle, 1985). Although not capable of forming a lamellar phase on its own, cholesterol readily forms a complex with the membrane phospholipids both by nonpolar (hydrophobic) interactions and by hydrogen bonding (Franks, 1976; Yeagle, 1985). A direct interaction of cholesterol with membrane proteins, evident from its effect on human erythrocyte band 3 and glycophorin, has also been considered a means by which cholesterol may modulate the functional activity of membrane proteins (Yeagle, 1985).

The content of cholesterol in membranes is variable, with the human erythrocyte membrane containing upward of 45 mole% of the total lipids, the endoplasmic reticulum about 10 mole%, and mitochondrial membranes relatively low levels (Cooper and Strauss, 1984; Yeagle, 1985). The fact that cholesterol occurs in variable amounts in different mammalian membranes, together with its complete absence in bacterial membranes, appears rather paradoxical considering its established functional role in some cellular membranes. This paradox is probably explicable by the fact that, although a certain level of fluidity is required for optimal functioning of cell membranes, it need not be the same in all membranes, and cholesterol may not be required to achieve this optimal level. In addition to the amount of cholesterol in the membrane, the transbilayer distribution of cholesterol and the association of cholesterol with various domains of membrane lipids that may interact with particular membrane proteins (Warren et al., 1975), are contributing factors in increasing overall heterogeneity of membrane lipid organization.

The phospholipid head group composition, the fatty acyl chain profile, and the cholesterol content of membranes are specific to each membrane, the tissue origin of that membrane, and the animal species from which the membrane is derived (Stubbs and Smith, 1984). With regard to the asymmetric distribution of membrane lipids, there appears a definite pattern of the class of phospholipids favored in a particular half of the membrane bilayer (Op den Kamp, 1979), although its functional significance is not clearly established. A slow rate of transmembrane diffusion (flip-flop) of phospholipids may assist in the maintenance of an asymmetric lipid bilayer (Kornberg and McConnell, 1971; Rousselet et al., 1976). Different phospholipid classes also display preference for certain types of fatty acids; e.g., cardiolipin is characterized by a relatively high proportion of 18:2 n-6 (Daum, 1985). The above features tend to counteract the potentially large number of compositional combinations and spatial orientations that membrane lipids could assume were only random structural and assembly processes operative.

[1]The nomenclature for fatty acid identification refers to the chain length and the number of double bonds; the number of carbon atoms from the last double bond to the terminal methyl end of the molecule is given by n-*x*. Trivial names are given in preference to the systematic name.

## Membrane Fluidity

The major intrinsic determinants of membrane fluidity[2] are the nature, arrangement, and interactions of the different molecular species of lipid present in the membrane. It is quite conceivable that a stable lipid bilayer structure possessing all the properties required of a permeability barrier could be achieved with far less variation in membrane lipid composition. However, the membrane must also support many of the processes essential for cell function. With the exception of certain lipids, such as the products of arachidonic acid metabolism and (poly)phosphatidylinositols (Hanahan and Nelson, 1984), these processes are performed by the membrane-associated proteins. Membrane proteins may be influenced by the physicochemical properties of lipid domains with which they may preferentially associate or by the integrative effects of all the membrane lipids. This physicochemical influence has been identified under an umbrella of terms, such as fluidity, microviscosity, order, disorder, rigidity, and lipid physical state. Furthermore, membrane fluidity contributes to, or is influenced by, processes such as homeoviscous adaptation, viscotropic regulation, lipid phase transitions, disorder⇌order transitions, and lateral phase separations (Jacobson and Papahadjopoulos, 1975; Quinn, 1981; Stubbs, 1983). The above terms describe rather than define some property of the hydrocarbon region of the membrane. Dietary lipids have the potential to alter the major intrinsic determinants of membrane fluidity and in turn influence membrane function. Before considering these, it is pertinent to review briefly the concept of membrane fluidity, although this has been covered recently in a number of excellent reviews (Quinn, 1981; Stubbs, 1983; Shinitzky, 1984a; Stubbs and Smith, 1984).

Segmental motions of the fatty acyl chains within the hydrocarbon core of the membrane bilayer are the major contributors to membrane fluidity. The flexibility of these chains results from the trans-gauche isomerizations, with both the range and rate of chain movement being important (Stubbs, 1983). Physical techniques have been used to measure these parameters and establish that a fluidity gradient exists in the bilayer, with fluidity increasing toward the methyl end of each fatty acyl chain (Hubbell and McConnell, 1971; Seelig and Seelig, 1974). Thus the central hydrocarbon region of the membrane bilayer is the most disordered. The cone of rotational motion of the fatty acyl chains is increased upon the introduction of a cis double bond, particularly when located in the middle of the acyl chain, as is the case for oleic acid (18:1 n-9). Disorder decreases as the double bond moves from the center toward either end of the acyl chain (Berde et al., 1980).

The motional characteristics of the hydrocarbon chains are also influenced when the lipids undergo a thermotropic phase transition (Quinn, 1981). Below the phase transition temperature ($T_c$), hydrocarbon chains exhibit restricted motion and pack closer, and the thickness of the bilayer increases. The opposite occurs above $T_c$, when membrane lipids are in the "liquid-crystalline" phase. In the heterogeneous mixture of lipids that mammalian membranes comprise, the lipid phase transition covers a broad temperature range within which lipids may coexist in different physical states (McElhaney, 1982). However, for most mammalian membranes, the composition of the membrane lipids is such that the vast majority of lipids would be expected to be above their characteristic phase-transition temperature at body temperature (37°C). The exception would be any small proportion of phospholipids with a disaturated fatty acid profile similar to those phospholipid molecular species observed in lung surfactant lipids (Possmayer, 1982), or perhaps certain molecular species of sphingomyelin (Barenholz, 1984). Within the liquid-crystalline state, differences in membrane fluidity could exist between membranes of

[2]For descriptive purposes only, membrane fluidity is considered as the reciprocal of membrane microviscosity.

different lipid compositions since the relative motion of the hydrocarbon chains would still be subject to those intrinsic characteristics that determine overall fluidity, i.e., chain length, degree and type of unsaturation, cholesterol content, and acyl chain distribution and position on the various classes of membrane phospholipids. The complexities of membrane architecture (see above) could add considerable heterogeneity to the lipid fluidity within the dimensions of the membrane. The asymmetric distribution of phospholipids and fatty acyl chains across the bilayer could lead to differences in the lipid fluidity in each bilayer half (Wisnieski and Iwata, 1977; Schroeder, 1980). The existence of lipid domains around membrane proteins could further add to the probability that lipid fluidity is not uniform within all regions of the membrane (Jain and White, 1977).

Evidence that mammalian lipids are normally in a liquid-crystalline state has come with the use of various physical techniques, particularly those employing spin or fluorescent probes to determine the motional characteristics of the membrane lipids. By anchoring and aligning a probe molecule alongside the native fatty acyl chain, comparisons can be made of the degree of rotational motion along the hydrocarbon chain (Hubbell and McConnell, 1971; Azzi, 1975; Thulburn et al., 1978). Thus the relative fluidity of different membranes resulting from alterations in lipid composition can also be measured. However, when using these techniques to determine membrane properties, some inherent problems must be recognized. Reporter molecules can potentially perturb the system under study. The location of the probe within the membrane is not accurately known. The probe molecule may preferentially reside in and report on distinct but unidentifiable regions within the membrane. Finally, it is the motional characteristics of the reporter molecules themselves that are measured rather than the motional characteristics of the native lipids (Keith et al., 1973; Azzi, 1975). Nevertheless, nonperturbing nuclear magnetic resonance (NMR) techniques have confirmed many of the observations obtained when using spin-labeled or fluorescent probes (Smith and Oldfield, 1984; Stockton and Smith, 1976; Bloom et al., 1978; Stubbs, 1983). In some instances, however, results using NMR have disagreed with those obtained using probe techniques, particularly regarding the concept of boundary or annular lipids associated with various membrane proteins (Jost et al., 1973; Marsh and Watts, 1982). However, the widely different time frame within which these different physical techniques probe molecular interactions must always be considered (Watts, 1981).

Despite the inability to understand completely and measure precisely many aspects of membrane fluidity, the major contributing factors to the level of fluidity are known. Such factors can be intrinsic and extrinsic, both of which can be interactive. Within the former category, the composition of membrane lipids is of primary importance (Quinn, 1981; Shinitzky, 1984a; Stubbs and Smith, 1984). However, the ratio of protein to lipid in the membrane is an important and often an overlooked determinant of membrane fluidity (Di Costanzo et al., 1983). Although different cellular membranes are characterized by a distinct protein to lipid ratio and a unique profile of marker enzyme activities, there is increasing evidence, particularly from dietary lipid studies, that the value of this ratio can be altered, probably as a result of changes in the absolute amount of lipid within the membrane. This alteration can subsequently influence membrane fluidity (Brasitus et al., 1985; Morson and Clandinin, 1985; Thomson et al., 1986; McMurchie, 1986; McMurchie, et al., 1987, 1988). Membrane fluidity appears to be inversely related to the protein to lipid ratio of the membrane (Shinitzky, 1984a).

Cholesterol plays an important role in membrane function, evidenced by the fact that changes in cholesterol levels (or the cholesterol to phospholipid ratio) dramatically influence many membrane-related activities, such as permeability (Demel et al.,

1972) and enzyme and receptor function (Demel and de Kruijff, 1976). Cholesterol has a variety of effects on the motional characteristics of the fatty acyl chains. For phospholipids in the liquid-crystalline state, an increase in cholesterol content significantly decreases fatty acyl chain mobility, and an intermediate fluid state is formed (Stockton and Smith, 1976; van Dijck et al., 1976). Therefore, changes in membrane cholesterol appear to be inversely related to membrane lipid fluidity (Cooper and Strauss, 1984; Yeagle, 1985). Membrane cholesterol can be manipulated by dietary as well as by in vitro means, with both increases and decreases being possible depending on the mode of treatment and the type of membrane. Because of its dramatic effect on membrane fluidity and function, there is a critical requirement for an efficient homeostatic mechanism to control membrane cholesterol content and to maintain a constant level of membrane fluidity (Cooper and Strauss, 1984; Yeagle, 1985), particularly in the face of potential perturbation by dietary lipids.

As determinants of membrane lipid fluidity, phospholipids can be either "fluidizers," e.g., phosphatidylcholine, or "rigidifiers," e.g., sphingomyelin. Thus the sphingomyelin to phosphatidylcholine ratio is inversely related to membrane lipid fluidity (Barenholz, 1984). As these two lipids are concentrated mainly in the outer half of the membrane bilayer, particularly in cell surface membranes (Op den Kamp, 1979; Barenholz, 1984), change in the sphingomyelin to phosphatidylcholine ratio may have its most prominent effect on membrane fluidity in that region of the membrane bilayer. Whereas changes in this ratio have profound influence on the surface-tension-lowering properties of pulmonary surfactant, particularly at birth (Possmayer, 1982), little scope is available in dietary lipid studies to deliberately alter it in most membranes. In the same manner, alterations in the proportions of the various phospholipid classes are also difficult to achieve directly, although some studies have shown alterations in response to differing dietary fatty acid supplements (Clandinin et al., 1985). The extent of membrane lipid manipulation achieved in mammalian membranes by dietary means does not appear as great, regarding the type of change or its magnitude, in comparison with the changes obtained using cell culture techniques or in vitro manipulation of the lipid composition of isolated membranes (Sinensky et al., 1979; Mahoney et al., 1980; Lochner et al., 1984; Stubbs and Smith, 1984).

Alterations in the phospholipid acyl fatty acid composition of mammalian membranes are readily achievable by dietary lipid supplementation. This avenue for modification is of particular importance in view of the fact that many characteristics of the fatty acyl chains influence membrane lipid fluidity. Fatty acyl chain length, degree and type of unsaturation, and mode of attachment and positional selection of acyl fatty acids onto the various phospholipids are all important determinants of membrane fluidity. In general, an increase in lipid unsaturation increases membrane fluidity (Quinn, 1981; Stubbs, 1983; Stubbs and Smith, 1984). However, increasing the number of cis double bonds beyond the first double bond does not induce a corresponding proportional increase in fluidity (Stubbs et al., 1981; Coolbear et al., 1983). This is relevant since the polyunsaturated nature of mammalian membrane lipids is maintained in the face of dietary lipid supplementation (Stubbs and Smith, 1984). The unsaturated acyl fatty acids that in vivo are above their gel to liquid-crystalline phase transition temperature contribute to the fluid nature of the membrane hydrocarbon region. The saturated fatty acids, mainly 16:0 and 18:0, tend to decrease membrane lipid fluidity. Thus the proportion of saturated to cis unsaturated fatty acids is inversely related to membrane fluidity (Quinn, 1981; Stubbs, 1983). In theory, changes in the value of this ratio should be achievable by dietary means, given the wide range of lipid saturation possible in dietary lipid supplements. However, in practice, a change in the ratio achievable in some instances never strongly reflects the extreme range of ratios

possible in the dietary lipid supplements. This highlights the efficiency of the homeostatic mechanisms responsible for maintaining a constant level of membrane fluidity in the face of potential perturbations resulting from dietary lipids. Failure of this homeostatic mechanism to restore membrane fluidity to the optimal or the original level could conceivably alter membrane function to the detriment of the organism. Such a situation may ultimately underlie some of the nutritionally based disorders discussed in the Introduction.

Membrane fluidity can also be influenced by a variety of extrinsic factors, including temperature, pressure, membrane potential, pH, and presence of divalent cations, particularly calcium (Papahadjopoulos, 1978). The interaction of these exogenous factors with the membrane will depend on the lipid composition, with dietary-induced changes having the potential to alter these interactions. The influence of temperature on membrane physical properties is dependent on the composition of the lipids, with the cholesterol content and degree of lipid unsaturation affecting the enthalpy and the critical temperature for phase transitions (Oldfield and Chapman, 1972; McElhaney, 1982). This would be a consideration when fluctuations in body temperature occur during fever, hypothermia, or hibernation (see chapters by Hazel and Aloia, this volume). Finally, many compounds can preferentially partition into the lipophilic region of the membrane and exert part of their biological effects by interacting with the lipids and thereby influence membrane function in a variety of ways (Goldstein, 1984). Included in this category are various classes of anesthetics, antiarrhythmics, antidepressants, hallucinogenic drugs, and ethanol. Therefore, dietary-mediated changes in membrane lipid fluidity may alter the interaction of these compounds with their site of action within the membrane lipid bilayer.

## Lipid Modulation of Membrane Proteins

Membrane proteins can potentially interact with any number of the vast array of lipid species that make up mammalian membranes. Alternatively, they may interact preferentially with either a particular species of lipid or a particular domain of lipid. Domains of lipid (Jost et al., 1973) may even buffer proteins from the effect of physical changes occurring in other regions of the membrane. Dietary modification of membrane lipid properties may result in an increase or decrease in some membrane functional activity, or some function may be made more or less responsive to an activator or inhibitor (Zsigmond and Clandinin, 1986). Such a result indicates that the membrane function has been modulated (Quinn, 1981).

The manner in which changes in fluidity influence membrane proteins, and hence modulate membrane activity, depends on a number of factors, including the particular protein, its equilibrium position in the membrane, the type of lipids in close proximity to the protein, and the manner in which the protein expresses functional activity, e.g., by lateral diffusion in the plane of the membrane. Apart from those membrane proteins only peripherally associated with the membrane or anchored by cytoskeletal elements, integral membrane proteins can undergo a number of alterations as a result of changes in membrane lipid fluidity. A protein may assume a new position in either the vertical or lateral plane of the membrane; the rate of lateral diffusion of a protein may be altered by a change in membrane lipid microviscosity; or, a protein may undergo some form of conformational change at its active site (Shinitzky, 1984b).

The position of a protein in the membrane is a reflection of its equilibrium state, which results from the maximization of the possible interactions it can undergo within the lipid and the aqueous regions (van der Meer, 1984). This equilibrium position may be disturbed when the membrane lipid composition is altered by dietary means. A change in phospholipid head group composition may alter electrostatic interactions between charged regions on the protein surface and the phospholipid head groups. A change in the

properties of the hydrocarbon region may alter the average free volume of the fatty acyl chains and the balance of the hydrophobic and electrostatic interactions between the membrane proteins, the water phase, and the lipid phase (van der Meer, 1984). Singularly or together, the above changes may displace membrane proteins to a new equilibrium position. This form of "passive modulation" is entropy-driven and will affect membrane proteins differently, depending, for example, on their original position in the bilayer (Shinitzky, 1984a). Changes in these interactions may be sufficient to induce vertical displacement of membrane proteins (Shinitzky, 1984b). Indeed, vertical displacement may be so dramatic that it leads to the shedding of proteins from the membrane (Borochov and Shinitzky, 1976).

The rotational motion and lateral diffusion of membrane proteins may also be affected by the physical properties of the membrane lipid phase (Shinitzky, 1984a). The rotation of a membrane protein in an axis perpendicular to the membrane plane may be affected predominantly by the immediate environment around that protein. Lateral diffusion of proteins in the plane of the membrane appears to be related to membrane lipid microviscosity and is important when microaggregation of receptors and other transducer components is a requisite for activation (Houslay, 1981). Conformational changes, which can potentially affect either an active or an allosteric site on the protein, may be induced by alterations in the lateral pressure within the membrane resulting from changes in the average free volume of the fatty acyl chains (van der Meer, 1984). Such changes may also occur when some of the membrane lipids no longer assume a liquid-crystalline phase structure. Lateral phase separations of lipids within the membrane during a broad phase transition can also influence the physical incorporation of proteins into membranes (Epand and Surewicz, 1984).

The above examples represent types of changes membrane proteins may undergo in response to altered membrane fluidity. However, similar changes in membrane fluidity may have opposite effects on the function of membrane proteins. Furthermore, any one function may exhibit a biphasic response to a change in membrane fluidity. This observation has led to the "optimum fluidity hypothesis," in which the activity of a particular membrane function requires an optimum fluidity level for maximum expression (Shinitzky, 1984a). This mechanism could allow for the "fine tuning" of functional activities by selective changes in membrane fluidity. The process of "homeoviscous adaptation," which restores membrane fluidity following perturbation (Sinensky, 1974), is also an important mechanism protecting membrane functions from adverse fluidity changes. It emphasizes the fact that, although the perturbation is initially localized to changes in membrane fluidity, it is the subsequent effects on membrane function that may ultimately be deleterious to the organism. For cell function to be maintained in the face of potential perturbation by dietary lipids, there must be an efficient mechanism available to maintain preexisting levels of membrane fluidity.

Many membrane-associated functions involving enzymes, receptors, and ion channels have been shown to be modulated by changes in membrane fluidity (Sandermann, 1978; Quinn, 1981; Stubbs and Smith, 1984). Although the actual molecular mechanism for modulation is unknown, it is likely that changes in the conformation, position or diffusion of protein(s) within the membrane, as discussed above, are involved. A distinction between those membrane-associated proteins that require the presence of lipid for functional activity (lipid dependence) and those lipid-dependent functions that can be altered by changes in the properties of the lipids with which they are associated (lipid modulation) may be solely a function of the methods employed to demonstrate these properties. Lipid dependence can best be demonstrated using reconstitution procedures in which a membrane protein is removed from its host environment, usually by deter-

gent treatment. If the subsequent loss of activity can be restored by addition of suitable lipids, then the protein activity is lipid-dependent (Buckland et al., 1981; Levitzki, 1985). In these types of experiments, the functional activity, although dependent on lipids, can sometimes be altered (modulated) by changing the type of lipids with which the reconstitution is performed (Etémadi, 1985; Levitzki, 1985). However, the act of removing intrinsic proteins from their surrounding membrane lipids, valid for demonstrating lipid dependence, may not be as valid for demonstrating lipid modulation.

Lipid modulation of membrane function is best demonstrated using in vivo or in vitro techniques that do not involve gross disruption to existing lipid–protein interactions in the native membrane. Modification of the lipid composition, as indicated previously, can induce a change in membrane fluidity sufficient to modulate the function of a membrane protein. Lipid compositional changes can be conveniently achieved by dietary means or by manipulating the lipid composition of the media of various cultured cell lines of animal origin. Alternatively, membrane lipid modifications can be made in vitro by selectively altering the lipid composition of isolated membranes without gross disruption. The use of phospholipid exchange proteins or the incubation of isolated membranes with selected mixtures of phospholipids, such as "active lipid" (Lyte and Shinitzky, 1985) or phospholipid-cholesterol mixtures, may alter membrane phospholipid composition or the cholesterol to phospholipid ratio. Cholesterol hemisuccinate has been used as a means of increasing the membrane cholesterol content (O'Connor et al., 1984). Modulation of membrane function by alterations in membrane fluidity can also be achieved by relying on physical changes induced by extrinsic factors. As was mentioned above, membrane lipids can undergo thermotropic disorder $\rightleftharpoons$order transitions, which provide a means to investigate the effect of altered lipid physical properties on membrane function. Measurement of the effect of temperature on the activity of membrane-associated enzymes, and subsequent presentation of data in the form of Arrhenius plots, has been widely used (Raison, 1973a,b), particularly in relation to dietary lipid studies (McMurchie and Raison, 1979; McMurchie et al., 1983a,c; Abeywardena et al., 1984). However, because the interpretation of Arrhenius plots still engenders considerable debate (Quinn and Williams, 1983), some comment is justified regarding their validity in demonstrating lipid modulation of membrane activity, particularly in dietary lipid studies.

When graphed as an Arrhenius plot, the temperature-activity profile of many membrane-associated enzymes is nonlinear (Raison, 1973a,b). Changes in slope, i.e., Arrhenius activation energy ($E_a$), occur at some characteristic temperature ($T_c$), which for mammals is usually around 20°C (McMurchie et al., 1973). In the temperature region above $T_c$, values for $E_a$ are usually lower than in the region below $T_c$. For some membrane enzymes, the temperature at which $T_c$ occurs can be altered by changing the membrane lipid composition, with dietary lipid supplementation being one such means (McMurchie and Raison, 1979; McMurchie et al., 1983a,c). The inverse relationship observed between $T_c$ and the value of $E_a$ when $T_c$ is altered indicates that some physical property of the membrane lipids related to the value of $T_c$ is proportional to the $E_a$ of the active site or rate-limiting step of the membrane enzyme system (McMurchie and Raison, 1979; McMurchie et al., 1983a,c; see also Aloia, this volume). Evidence that the change in $E_a$ of various membrane enzymes is related to some form of temperature-induced change in the physical properties of the membrane lipids has come from parallel studies using spin-labeled or fluorescent probes. When infused into the native membrane or into aqueous dispersions of isolated membrane lipids, some motional parameter of the probe molecule changes abruptly at a temperature coincident with the $T_c$ observed for membrane enzyme activity (McMurchie et al., 1973; Raison, 1973a,b). The "break"

in the Arrhenius plot for various membrane enzymes can be abolished by treatments designed to disrupt lipid–lipid and/or lipid–protein interactions, such as the addition of nonionic detergents (Raison, 1973a,b; McMurchie and Raison, 1979). For some membrane-associated enzymes, such as the mitochondrial electron transport chain of chilling-resistant plants and poikilothermic vertebrates, Arrhenius plots of activity are linear (i.e., they display a constant $E_a$ in the temperature range from 0°C to about 35°C), and no corresponding abrupt change in the molecular ordering of the lipids is observed using spin-labeling or fluorescent probes (McMurchie et al., 1973; Raison, 1973a,b). This result, which is in contrast to results observed for the same enzyme activities in chilling-sensitive plants and homeothermic animals, is explicable in terms of differences in mitochondrial membrane lipid composition that presumably lead to differences in membrane lipid fluidity between the two groups. For the chilling-resistant plants and the poikilothermic vertebrates, it is believed that the temperature-induced change in the membrane lipids influencing mitochondrial electron transport activity occurs at some temperature below 0°C (McMurchie et al., 1973; Raison, 1973a,b).

The use and interpretation of Arrhenius plots for demonstrating lipid modulation of membrane function have been questioned on a number of grounds. Some of the arguments raised include the following: 1) The abrupt change in $E_a$ is due to a direct effect of temperature on the membrane protein(s) and is independent of effects originating from the membrane lipids (Stanley and Luzio, 1978; Madden and Quinn, 1979; Bligny et al., 1985). 2) From the type of lipids present in most mammalian membranes, a thermotropic lipid phase transition would not be observed in the membrane lipids at temperatures corresponding to the "break" in the Arrhenius plot of membrane enzyme activity (Quinn and Williams, 1983). 3) The reporter molecules used in parallel biophysical studies may perturb the membrane lipid region they probe and may preferentially reside in, and report on, a discrete region of the membrane exhibiting unique physical properties (Keith et al., 1973; Schreier et al., 1978). 4) Arrhenius plots are best represented as being curvilinear rather than as exhibiting abrupt "breaks" (Bagnall and Wolfe, 1978). 5) "Breaks" in Arrhenius plots of membrane enzyme activity may be an artifact of the assay system resulting from temperature-induced changes in substrate affinity (Silvius et al., 1978).

Evidence from some studies suggests that temperature has a direct (i.e., nonlipid-mediated) effect on the temperature dependence of the $E_a$ of various membrane enzymes, particularly some ATPases (Johannsson et al., 1981; East et al., 1984). However, for other enzyme systems, most notably the mitochondrial electron transport system (Raison, 1973a,b; McMurchie et al., 1983a–c) and hormone-sensitive adenylate cyclases (Sinensky et al., 1979; Houslay, 1985), the evidence clearly supports the notion that the "break" in the Arrhenius plot most likely is due to some lipid-mediated event. The actual membrane lipids responsible and the mechanism by which they induce a change in the value of the $E_a$ of a membrane enzyme are not known. Mammalian membrane lipids are highly unsaturated, and, from the acyl chain distribution, there is a low probability that a disaturated phospholipid species exhibiting a relatively high phase transition temperature would exist in most membranes. Nevertheless, if present, such a phospholipid species could conceivably undergo a thermotropic phase transition at or near the characteristic "break" temperature and form a small "solidus" domain within the predominant liquid-crystalline membrane phase. Thermotropic phase transitions of a relatively small enthalpy value have been detected in mammalian mitochondrial membrane lipids by sensitive calorimetric techniques at temperatures of about 20°C (Blazyk and Newman, 1980; McMurchie et al., 1983b).

For *Acholeplasma laidlawii* cell membranes, which possess a far less complex

array of lipids than mammalian membranes, the temperature at which a "break" is observed in membrane-associated $Mg^{2+}$-ATPase activity correlates with the thermotropic phase transition of the membrane lipids as determined by calorimetry (McElhaney, 1986). This correlation is maintained following extensive manipulation of the cell membrane lipid composition (Silvius and McElhaney, 1980). $Mg^{2+}$-ATPase activity from *A. laidlawii* cell membranes and succinate cytochrome c reductase activity from rat heart mitochondria have also been analyzed by assuming that the respective Arrhenius plots displayed either an abrupt "break" or, alternatively, were curvilinear. Analyzing the data by either method revealed similar characteristic temperatures for each enzyme activity (Silvius and McElhaney, 1980; McMurchie et al., 1983a). Overall from those studies in which thermotropic changes in the properties of the membrane lipids have been used to induce changes in membrane enzyme activity, there is sufficient evidence to reinforce the concept that the physical properties of the membrane lipids can modulate some functional activities of the membrane proteins.

Extrinsic factors can also influence membrane activity. For example, lipid-soluble compounds that perturb membrane lipid fluidity, such as benzyl alcohol, can influence a number of membrane-associated enzyme activities, including the hormone-sensitive adenylyl cyclases (Gordon et al., 1980). When both intrinsic and extrinsic factors are combined to alter membrane lipid fluidity, as is achieved by altering membrane lipid composition in concert with changes in temperature, there is little doubt that, although not uniform or universal, the phenomenon of lipid modulation of membrane protein function exists. This modulation has wide biochemical and physiological implications at the cell, tissue, and organism levels. The existence of powerful homeostatic mechanisms for the maintenance of appropriate levels of membrane fluidity further reinforces the important regulatory role that membrane fluidity plays in cell function (Thompson and Martin, 1984; Thompson, 1986).

## MEMBRANE LIPID METABOLISM AND REGULATION OF MEMBRANE FLUIDITY

The strategy for nutritional investigations of membrane function relies on the fact that the lipid composition of cell membranes can be altered, even when the diet is adequate in all nutrients. Besides the effect of dietary lipids, cellular lipid synthesis can also contribute to the membrane lipid composition. The potential compositional changes that membranes can undergo include alterations in the cholesterol to phospholipid ratio, in the phospholipid class distribution, in the type of fatty acids (chain length and degree of unsaturation), and in the combination of these acyl fatty acids on the membrane phospholipids. Phospholipid composition may be altered in a gross manner during synthesis, inducing large changes in membrane fluidity, or, alternatively, in situ, allowing for the fine tuning of membrane fluidity (Thompson and Martin, 1984; Thompson, 1986). Factors that could influence changes in the membrane lipid composition include the contribution of the dietary lipid pool, size and composition of the lipid substrate pools, fatty acyl chain elongation and desaturation activity, and the extent to which membrane lipid composition and fluidity are perturbed by exogenous influences.

### Synthesis and Desaturation of Fatty Acids

The primary product of de novo fatty acid biosynthesis in mammals is palmitic acid (16:0), formed initially by the action of acetyl-coenzyme A (CoA) carboxylase on acetyl-CoA, forming malonyl-CoA. Upon the action of a cytosolic fatty acid synthetase complex involving an acyl carrier protein, free fatty acids are released by an acyl thioesterase. These fatty acids and those derived from dietary sources are acted upon by a fatty acyl-CoA synthetase to form the fatty acyl-

CoA derivative, which is then available for chain elongation and desaturation before being incorporated into structural lipids. Studies on the fatty acyl-CoA synthetase in the endoplasmic reticulum have established that this enzyme complex is a major regulatory point in the production of fatty acyl-CoA derivatives for incorporation into membrane phospholipids (Jeffcoat and James, 1984; see Lynch and Thompson, this volume).

Fatty acyl chain desaturation and elongation are necessary steps for the production of the polyunsaturated membrane lipids (Jeffcoat and James, 1984). The desaturation step is catalyzed by enzymes that exhibit a high specificity for the position at which a double bond is introduced into a fatty acyl-CoA chain. Desaturation of stearoyl-CoA by a Δ9-desaturase introduces a cis double bond between the ninth and tenth carbon atoms from the original carboxyl end, to form oleoyl-CoA. Desaturation is an oxygen-dependent, iron-requiring process, associated with the endoplasmic reticulum, which contains the desaturase enzyme and a short electron transport chain consisting of cytochrome $b_5$ and its reductase (Holloway and Holloway, 1975; Rogers and Strittmatter, 1975). Desaturation occurs toward the carboxyl (CoA) end of the fatty acyl chain. Because of the direction of desaturation, the first double bond nearest the terminal methyl end of linoleic acid (n-6 series) is at the Δ12 position (12 carbons from the carboxyl terminus), and that of linolenic acid (n-3 series) is at the Δ15 position. The lack of a desaturase enzyme in mammalian cells of the Δ12 or higher variety implies that linoleic and linolenic acids cannot be synthesized by the mammalian cell and must be obtained from the diet, (originally from plant sources). Both of these fatty acids, as CoA derivatives, are acted on by a Δ6-desaturase to add a third and fourth cis double bond, respectively. This maintains a total of six (n-6) and three (n-3) carbon atoms between the terminal methyl group and the nearest double bond, respectively, for each of these families of polyunsaturated fatty acids. By this mechanism, mammals are able to synthesize several noninterconvertible families of polyunsaturated fatty acids by successive chain elongation and desaturation catalyzed by Δ6-, Δ5-, and Δ4-desaturase reactions. The precursors for these reactions can be derived by either de novo synthesis, e.g., oleic acid (n-9), or from dietary sources, i.e., the n-6 (linoleic) and n-3 (linolenic) acid families. Therefore, the fatty acid composition of membrane lipids will be influenced by the availability of linoleic and linolenic acids from the diet. In their absence, the desaturase system will chain elongate and desaturate oleic acid to eicosatrienoic acid, 20:3 n-9 (Mead acid), thereby elevating the triene to tetraene ratio, which is considered a diagnostic marker for essential fatty acid deficiency (Holman, 1986). The supply of dietary polyunsaturated fatty acids controls desaturation of the saturated fatty acids and can therefore control fatty acid synthesis and triacylglycerol secretion by the liver (Jeffcoat and James, 1984).

The Δ6-desaturase step is another regulatory point for final production of the polyunsaturated fatty acids, such as arachidonic, docosapentaenoic, and docosahexaenoic acids (Brenner, 1977). A number of factors influence Δ6-desaturase activity. Evidence suggests that the lipid fluidity of the endoplasmic reticulum membrane may itself regulate the activity of this membrane-bound enzyme (Holloway and Holloway, 1975; Peluffo et al., 1976), although the requirement may be only for the enzyme to be in a fluid lipid environment. The Δ6-desaturase is inhibited by ageing, fasting, diabetes mellitus, glucose, ethanol, protein deficiency, saturated and trans fatty acids, and a number of hormones, including epinephrine, thyroxine, and glucocorticoids (Brenner, 1982). Furthermore, carnivores, such as cats, probably lack this enzyme, and the same may be true for eskimos (Sinclair, 1984). $Zn^{2+}$ is implicated as a cofactor for the Δ6-desaturase, whereas copper ions inhibit the enzyme (Wahle, 1983). The preference for desaturation by the Δ6-desaturase is in the order of

n-3 > n-6 > n-9 (Brenner and Peluffo, 1966).

## Assembly and Compositional Variations of Membrane Phospholipids

The synthesis and desaturation of fatty acids from both de novo and dietary sources provide a means of altering the type of (activated) fatty acid available for incorporation into membrane phospholipids. The positional specificity and the class of phospholipid to which fatty acyl chains are attached are important determinants of membrane fluidity. For example, differences in the head group composition of phospholipids have an effect on the phase transition temperature ($T_c$), with pure phosphatidylcholine having a $T_c$ 20–30°C lower than phosphatidylethanolamine for an identical fatty acyl composition (van Dijck et al., 1976). The potential diversity generated by the positional distribution of fatty acyl chains on the various classes of phospholipids is diminished by a number of intrinsic controls. Nonrandom positioning of fatty acyl chains on the phospholipids, as well as differences in the composition of the pools from which fatty acids are selected for incorporation, decreases compositional diversity of membrane phospholipids. Because of changes in the supply and demand for fatty acyl chains during phospholipid synthesis, and, indeed, after phospholipids have been incorporated into membranes, the physicochemical properties of membranes may still be subject to continuous, subtle changes by mechanisms including fatty acyl chain rearrangement and phospholipid head group exchange (Thompson and Martin, 1984).

Synthesis of mammalian membrane phospholipids requires fatty acyl-CoAs and glycerol-3-phosphate. The former is synthesized by the action of fatty acyl-CoA synthetase, and the latter arises from dihydroxyacetone phosphate or from glycerol. Acylation of two molecules of fatty acyl-CoA to glycerol-3-phosphate to yield the intermediate 1,2-diacylglycerol-3-phosphate (phosphatidic acid) occurs initially by the action of fatty acyl-CoA:*sn*-glycerol-3-phosphate acyltransferase (Bell and Coleman, 1980) to form lysophosphatidic acid, followed by the action of fatty acyl-CoA:1-acyl-*sn*-glycerol-3-phosphate acyltransferase. Phosphatidic acid can be hydrolyzed to form a 1,2-diacylglycerol by the action of phosphatidic acid phosphatase (Thompson and Martin, 1984). Phosphatidylethanolamine synthesis results from the ethanolamine phosphotransferase-catalyzed reaction between diacylglycerol and CDP-ethanolamine, the latter being synthesized from ethanolamine, ATP, and CTP. Two pathways occur for phosphatidlycholine synthesis. First, phosphatidylethanolamine can be converted to phosphatidylcholine by three methylation steps utilizing S-adenosyl methionine. A second pathway (the salvage pathway) utilizes choline released by the breakdown of phosphatidylcholine. This choline is formed into CDP-choline, which is available for reaction with diacylglycerol to form phosphatidylcholine. The salvage pathway is the preferred pathway for phosphatidylcholine synthesis in higher animals, with only about 20% of the molecular species originating from phosphatidylethanolamine being transferred to phosphatidylcholine by way of the methylation pathway in mammalian liver (Sundler and Akesson, 1975). The conversion of phosphatidic acid to CDP-diacylglycerol by CTP:diacylglycerolphosphate cytidylyltransferase, eventually gives rise to phosphatidylserine, phosphatidylinositol, phosphatidylglycerol, and cardiolipin. The rate of phosphatidylcholine biosynthesis is regulated by the activity of CTP:phosphocholine cytidylyltransferase (Choy et al., 1977).

Phospholipid synthesis occurs at a number of sites in the cell utilizing a variety of fatty acyl chains to complete the molecule. The predominant membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, are synthesized on endoplasmic reticular membranes, whereas cardiolipin, which is normally restricted to mitochondrial membranes, is assembled in that organelle (Quinn, 1981). As a possible reflection of the different sites of synthesis of these phospholipids

and the availability of particular fatty acids at these sites, together with the selectivity and distribution of the enzymes synthesizing the phospholipids (Lands, 1979), the acyl fatty acids of phosphatidylcholine and phosphatidylethanolamine show positional specificity with primarily saturated fatty acids at the *sn*-1 position and unsaturated fatty acids at the *sn*-2 position. Assembly of the fatty acyl chains on the various positions of cardiolipin appears to be a more random process (Stubbs and Smith, 1984; Thompson and Martin, 1984).

Retailoring of membrane phospholipids after their synthesis provides another mechanism for altering membrane physical properties (Thompson and Martin, 1984). Phospholipases $A_1$ and $A_2$ cleave a fatty acid from the *sn*-1 and *sn*-2 positions of a phospholipid, respectively, allowing the potential for reacylation with a different profile of fatty acids. Phospholipase $A_1$ is widely distributed in various cellular compartments, including the plasma membrane; however, in rat liver, it does not appear to be associated with the mitochondrion (van den Bosch, 1980). Cleavage of the saturated fatty acid at the *sn*-1 position is not affected by the nature of the unsaturated fatty acid in the *sn*-2 position of the phospholipid (Holub, 1982). Phospholipase $A_2$ has been found as a membrane-associated enzyme in a number of cellular membranes, including the plasma membrane and the mitochondrion; however, only trace amounts are present in liver endoplasmic reticular membranes (van den Bosch, 1980). This phospholipase may be regulated by $Ca^{2+}$ ions and calmodulin (Wong and Cheung, 1979) and shows some specificity for the type of unsaturated fatty acid at the *sn*-2 position, with oleic, linoleic, linolenic, and arachidonic acids being cleaved preferentially in that order (Waite and Sisson, 1971). The physical properties of the phospholipid substrate for phospholipase $A_2$ markedly influence the activity of the enzyme, there being a marked increase in activity near the phase transition temperature of the phospholipid (Kannagi and Koizumi, 1979). This enzyme also plays an important role in releasing arachidonic acid from the *sn*-2 position of membrane phospholipids for synthesis of prostanoids and eicosanoids (Irvine, 1982).

Following phospholipase action, deacylated phospholipids are available for reacylation by the action of two fatty acyl transferases (fatty acyl-CoA, lysolipid acyl transferase), utilizing as substrates fatty acyl-CoAs derived from dietary or de novo sources and acylating in a specific manner at either the *sn*-1 and *sn*-2 positions. Compositional characteristics, such as fatty acyl chain length and degree of saturation and unsaturation, as well as the nature of the phospholipid head group, determine the specificity of these acylating enzymes (Lands, 1979). These factors, along with the substrate pool of fatty acyl-CoAs available for reacylation, account for the positional specificity and unique fatty acid profile observed with the various classes of phospholipids present in mammalian membranes. It is believed that the deacylation process probably limits the rate of acyl group turnover, whereas reacylation probably controls the specificity of the phospholipid retailoring process. In that deacylation is usually relatively rapid, a shift in acyltransferase specificity can quickly bring about a shift in the molecular species of phospholipids present in the membrane (Thompson and Martin, 1984). Such retailoring of existing fatty acyl components to give rise to different molecular species of phospholipids could alter membrane fluidity but the magnitude achievable would probably not be as great as that resulting from gross compositional changes in the acyl fatty acid profile. In the context of homeoviscous adaptation and viscotropic regulation of membrane fluidity (Stubbs, 1983), the changes resulting from in situ phospholipid retailoring compared with the compositional changes achievable during phospholipid synthesis may serve the function of accommodating small and large perturbations in membrane fluidity, respectively. Alternatively, these processes may reflect short- or long-term strategies for

the homeostasis of membrane fluidity (Thompson and Martin, 1984).

Phospholipid molecules may also be transferred in toto between differing cellular membranes during membrane fusion. Indeed, membrane flow from points of membrane synthesis, such as the endoplasmic reticulum, to peripheral locations, such as the plasma membrane, may occur via a process of intermittent membrane fusion involving a variety of different membrane types (Morré et al., 1984). Alternatively, membrane phospholipid composition may be changed by cytosolic, phospholipid exchange proteins that catalyze the direct transfer of phospholipid molecules between membranes without the need for membrane fusion (Wirtz, 1982). Although some phospholipid exchange proteins display specificity with regard to the class of phospholipid exchanged, and even for the relative fluidities of the donor and acceptor membranes (Read and Funkhouser, 1984), the net effect of the exchange process on the distribution of phospholipid classes in the membrane may not be great. However, because phospholipid exchange proteins do not exhibit great specificity with regard to the nature of the fatty acyl chains, considerable changes in the acyl fatty acid profile (and hence membrane fluidity) could occur via this mechanism.

## Membrane Cholesterol

Individuals in developed western societies normally consume between 300 and 600 mg of cholesterol per day (Kummerow, 1985). In mammals, esterified cholesterol is transported in the plasma in the form of lipoproteins which exhibit different cholesterol to phospholipid ratios and hydrated densities, with low-density lipoprotein (LDL) and high-density lipoprotein (HDL) being the major plasma lipoproteins of mammals (Chapman, 1980). Cholesterol can move into cells from plasma lipoproteins by exchange diffusion, pinocytosis, or receptor-mediated interactions involving endocytosis (Cooper and Strauss, 1984). The lipid composition of the surrounding lipoproteins greatly influences the cholesterol content of the red cell membrane, with the cholesterol to phospholipid ratio of both the membrane and the donor lipoprotein pool appearing to be the most important determinants in exchange diffusion reactions (Cooper and Strauss, 1984). The cholesterol to phospholipid ratio of human red blood cell membranes can be manipulated from 0.4 to 2.7 by changes in the equilibrium partitioning of both cholesterol and, mainly, phosphatidylcholine (Cooper et al., 1978). In contrast to cellular pinocytosis of lipoproteins, which is not specific for any one type of plasma lipoprotein, receptor-mediated uptake of lipoproteins into cells is lipoprotein specific, with the LDL receptor-mediated pathway being partly responsible for the uptake of circulating LDL-associated cholesterol by extrahepatic cells. Entry of cholesterol via the receptor pathway regulates both the amount of LDL receptor available on the cell surface and the extent of de novo sterol synthesis by the cell (Brown and Goldstein, 1986).

Cholesterol biosynthesis occurs by way of at least 30 discrete enzyme steps, with 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), located on the endoplasmic reticulum, being an important regulatory site in the cholesterol synthetic pathway (Rodwell et al., 1976). The level of HMG-CoA reductase is controlled by enzyme synthesis and degradation, and the activity is influenced by phosphorylation/dephosphorylation of the enzyme protein (Beg et al., 1978). Furthermore, HMG-CoA reductase is sensitive to multivalent feedback control, not only by cholesterol but also by intermediates of the overall synthetic pathway and their metabolites (Kandutsch et al., 1978; Brown and Goldstein, 1980). Excess cholesterol derived from the LDL pathway and biosynthesis stimulates cholesterol ester storage via the enzyme fatty acyl-CoA:cholesterol acyltransferase (Spector et al., 1979).

The amount of cholesterol available for membrane incorporation is controlled by factors such as the dietary cholesterol intake, intracellular synthesis and degradation, and

formation and hydrolysis of cholesterol esters. Dietary fatty acids will also influence the plasma lipoprotein levels, with hypo- and hypercholesterolemic effects on plasma cholesterol being observed with dietary polyunsaturated and saturated fatty acids, respectively (Grundy, 1979; Kummerow, 1985). Therefore, the influence of dietary lipids on membrane cholesterol is a complex process depending on a number of interactive factors operating at the dietary, circulating lipoprotein, and cellular levels. The lipid composition of the target membrane, the specificity of the "transporter," and the particular exchange mechanism for movement of cholesterol into and out of cellular membranes are all important in the final determination of the membrane cholesterol content. Disturbance to the steady state of the above mechanisms could alter membrane cholesterol content and, hence, membrane fluidity and function. If homeostatic mechanisms for membrane cholesterol are insufficient to correct or buffer against various perturbing influences, changes resulting from alterations in membrane fluidity could be detrimental to cell function in a manner similar to the effects of changes in membrane phospholipid composition (Shinitzky, 1984a,b; Cooper and Strauss, 1984).

Once incorporated into membranes, cholesterol can readily move from one cellular membrane to another by a process of vesicular exchange during membrane fusion or by transfer of cholesterol through the aqueous phase of the cell via sterol carrier mechanisms (Bell, 1978; North and Fleischer, 1983). The effect of cholesterol on membrane fluidity and function will be influenced by the nature and site of interaction of cholesterol with the membrane phospholipids or even membrane proteins. A nonuniform distribution of cholesterol either vertically or laterally in the membrane may have differential effects on lipid fluidity in various regions of the membrane. However, in contrast to membrane phospholipids, cholesterol appears to display a symmetric transbilayer distribution at least in the human red blood cell, which may be aided by a relatively high rate of transbilayer "flip-flop" (on the order of seconds; Lange et al., 1981). Finally, cholesterol may be preferentially included or excluded from various regions of the membrane phospholipids, which act as annular or boundary lipids around various membrane proteins (Warren et al., 1975; van Dijck et al., 1976; Johannsson et al., 1981).

## DIETARY SOURCES OF LIPID FOR MEMBRANE MODIFICATION

Mammalian membrane lipids are derived in part from the diet, and advantage can be taken of this fact in experiments designed to manipulate membrane lipid composition. As discussed above, mammalian cells require a dietary supply of the essential fatty acids linoleic (18:2 n-6) and α-linolenic (18:3 n-3) because of their inability to desaturate at certain positions along the acyl chain. These essential fatty acids are the precursors of independent, noninterconvertible families of polyunsaturated fatty acids (Holman, 1986) required for the structural membrane lipids and the synthesis of the chemically reactive lipids derived from arachidonic acid and phosphatidylinositol (Hanahan and Nelson, 1984). The pool of nonessential fatty acids (i.e., those derived by de novo lipid synthesis) can also be influenced by dietary consumption, since fatty acids within this group (e.g., palmitic, stearic, and oleic acids) are relatively abundant in the diets of many developed western populations.

Initial experiments concerning dietary modification of membrane lipid composition were aimed at highlighting the essential nature of certain polyunsaturated fatty acids, and these experiments relied on the exclusion of a particular (essential) polyunsaturated fatty acid (Williams et al., 1972; Bloj et al., 1973b). The second generation of experiments, which is ongoing, tend to use the essential nature of polyunsaturated fatty acids to explore effects of overnutrition on membrane function. These experiments focus on describing the role(s) of polyunsaturated fatty

acids as membrane structural components and as chemically active species of lipid. The third generation of dietary lipid experiments appears to have a more strategic goal in mind. These experiments, utilizing potentially harmful or beneficial dietary fats, are directed toward understanding their possible role in the etiology of various human disorders. The rationale is that certain disorders may be influenced by nutritional components and that a disturbance in cell membrane function is an underlying cause. Thus there has been a preponderance of dietary experiments in the area of heart disease and its possible relationship with nutritional factors. The roles of elevated n-3 polyunsaturated fats, reduced saturated fats, alterations in the dietary polyunsaturated to saturated (P/S) fatty acid ratio, total fat intake, and hypo- and hypercholesterolemic diets (Nestel et al., 1984; Arntzenius et al., 1985; Dyerberg, 1986) have, therefore, attracted considerable attention. Studies of possible detrimental effects (e.g., cardiotoxicity) when feeding unsaturated fatty acids, such as erucic acid (22:1 cis Δ13) in rapeseed oil and cetoleic acid (22:1 cis Δ11) in fish oil concentrates are other examples of recent mission orientated types of investigation (Bremer and Norum, 1982).

Attention has also been paid to the effect of structurally modified dietary fats, such as those containing trans, methyl, or ethyl side chain fatty acids (Beare-Rogers, 1983). Various food-processing procedures, including the hydrogenation of margarine, can markedly increase the content of trans unsaturated fatty acids in foods, whereas milk-fat-depressing diets can also elevate the content of trans fatty acids in milk (Wahle, 1983). As a result of these commercial practices, there is concern regarding the consumption of trans unsaturated fatty acids, considering that these fatty acids behave, at least in membranes, in a manner similar to saturated, straight-chain fatty acids (Hsu and Kummerow, 1977; Beare-Rogers, 1983).

Diets commonly used to modify membrane lipid composition are those providing a high or a low P/S ratio in combination with a high or a low level of dietary fat. In dietary experiments performed in our laboratory, the P/S ratio ranges between 0.16 and 3.77, with up to 40% of the caloric intake being in the form of fat (McMurchie et al., 1983a–c, 1986). Elevation of the dietary P/S ratio can be achieved by the addition of plant oils such as sunflower seed or safflower oil, both of which are high in the n-6 polyunsaturated fatty acid linoleic acid, although other plant oils could suffice (Sinclair, 1984). Some of these oils have been fed in a protected form to ruminant animals to prevent prior hydrogenation by rumen bacteria (Faichney et al., 1972). Depending on their source, fish oils are high in both the n-6 and n-3 families of polyunsaturated fatty acids, with the n-3 family being represented by eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3; Gibson, 1983). Dietary supplementation with n-3 polyunsaturated fatty acids derived from fish oils has recently found favor over the use of linseed oil supplements (α18:3 n-3), perhaps because of rancidity and taste considerations, together with the possibility that dietary intervention in human studies can be accomplished with fish and fish oil supplements. Other sources of polyunsaturated fats include oil of the evening primrose and black currant juice, both of which have a relatively high proportion of γ-linolenic acid (γ-18:3 n-6). Dietary supplementation with this fatty acid is designed to bypass the Δ6 desaturase reaction, which converts linoleic to γ-linolenic acid (Huang et al., 1984, 1985b), the rationale being that many factors are known to inhibit Δ6-desaturase activity (Brenner, 1982). In all dietary lipid studies involving supplementation with polyunsaturated fatty acids, antioxidants must be considered, carefully selected, and adequately controlled in the experiment. Although gavage or intragastric cannula techniques have been used as a means of feeding fatty acids to animals (Williams et al., 1975), ad libitum or pair feeding is usually practised.

Decreasing the P/S ratio of the diet has been achieved by supplementing low mixed-fat diets with palm or coconut oil with or

without prior hydrogenation. However, these oils contain a considerable proportion of shorter-chain, saturated fats not normally found in mammalian membrane phospholipids. Furthermore, hydrogenation can lead to production of trans unsaturated fatty acids (Beare-Rogers, 1983). Supplementing the diet with animal fats such as beef tallow, pig lard, or sheep kidney (perirenal) fat is probably the better means of increasing the proportion of saturated fatty acids and lowering the P/S ratio. These animal fats have high proportions of palmitic and stearic acids, virtually no short-chain, saturated fats, and, in most cases, sufficient linoleic and linolenic acids to overcome any possibility of inducing an essential fatty acid-deficient condition (McMurchie et al., 1983a–c). Although these animal fats exhibit a relatively high proportion of oleic acid, they do reflect the type of saturated fatty acids consumed by developed western societies (Kummerow, 1985).

Alteration in dietary cholesterol levels is easily achieved with addition of cholesterol, with or without added cholate as "carrier." However, the choice of the experimental animal model and the overall dietary fat loading will markedly influence plasma cholesterol levels. For example, in the rat, 2% dietary cholesterol has little effect on plasma cholesterol levels, even in combination with high fat loading (McMurchie et al., 1987). However, in the marmoset monkey, which we have extensively used for dietary lipid experimentation, plasma cholesterol levels may increase from about 180 mg/100 ml in control (low mixed fat) fed animals to about 480 mg/100 ml with 0.5% cholesterol loading, to well over 1,300 mg/100 ml when the level of fat supplement in the diet reaches 16% irrespective of the nature of the fat supplement (McMurchie et al., 1988).

## MODIFICATION OF MEMBRANES BY DIETARY LIPIDS

The effect of changes in membrane lipid fluidity on a variety of cell processes, including membrane-associated enzyme, receptor, and carrier-mediated transport activities, has been extensively reviewed by Shinitzky (1984a). His review describes the extent to which important membrane functions are dependent on the fluidity/microviscosity of the membrane lipids. However, apart from studies by Brivio-Haugland et al. (1976) and McMurchie and Raison (1979), which are reviewed below, his information has been compiled from in vitro lipid modification using isolated cells and membranes as well as membrane lipid manipulations using cell culture and reconstitution techniques rather than from dietary studies of the type that are the subject of this review.

In the context of dietary lipid regulation of membrane fluidity and function, ideally, the following aspects should be examined in the one study: the lipid composition of the membrane following dietary lipid supplementation, the effect of compositional changes on membrane fluidity determined by an appropriate biophysical technique, and the measurement of membrane functional activity together with unequivocal evidence that any observed functional change was due to some modification of the membrane lipids. Additionally, consideration must be given to possible differences resulting from both chronic vs. acute dietary lipid manipulation and compensatory homeostatic changes, which could complicate the interpretation of results.

The common theme for all studies reviewed in this section is an altered fat intake by the particular animal species under investigation. Both the amount and type of dietary fat can be altered, as was discussed in the previous section. In most studies, some form of lipid analysis, usually of the isolated membrane, is undertaken. However, at this point, the number of studies that also measure both membrane functional activity and physical properties decreases. When the measurement of membrane function has been combined only with membrane lipid analysis, it is often argued that the observed functional changes were due to some change in membrane fluidity. These arguments are usually based on the known lipid-dependence of a particular

membrane function and the fact that the diet would have induced some physicochemical change in the properties of the membrane lipids because of an altered membrane lipid composition. These studies are often sufficient to establish with some confidence that dietary lipids can modulate membrane functional activity. However, the existence of such an effect would be better confirmed if some aspect of the membrane physical properties was also studied and if evidence was presented of the mechanism involved in dietary lipid modulation. Therefore, the extent to which the above aspects have been studied provides a convenient selection criterion to categorize the many studies on dietary lipids and their effect on membrane composition and function.

### Membrane Lipid Composition

A summary of a number of studies on the effect of dietary lipids on membrane lipid composition in experimental animals and man is given in Table 7–1. Most of the studies report changes in the lipid composition of mitochondrial and microsomal membranes from rat tissue, particularly liver and heart. Although a wide range of dietary lipid supplements has been used, and many facets of the influence of diet on membrane lipid composition have been studied, some generalizations can be made on the effect dietary lipids exert on membrane lipid composition and how such changes may potentially influence membrane lipid fluidity.

Despite changes in the proportion of individual saturated and unsaturated fatty acids in the membrane resulting from dietary lipid supplementation, the proportion of saturated to unsaturated fatty acids remains remarkedly constant in the face of large variations in the dietary intake of saturated and unsaturated fats (Gibson et al., 1984; Charnock et al., 1985c). Under these conditions, membrane lipids also maintain a relatively constant value for their unsaturation index (Tahin et al., 1981; Stubbs and Smith, 1984). These effects are evident for membranes from different cellular and tissue locations that initially display wide variations in lipid composition. Conclusions drawn from observations in the rat also held for other animal species, including marmoset monkeys (Charnock et al., 1985a; McMurchie et al., 1986, 1988). These results indicate that it is unlikely that major changes in membrane fluidity occur in vivo, resulting from changes in the degree of membrane lipid saturation via dietary fat supplementation. This conclusion is reinforced by the observation that the predominant membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, still retain selective fatty acyl chain positioning, i.e., *sn*-1 (saturated) and *sn*-2 (unsaturated), in the face of extreme variation in the dietary fat intake (Iritani and Fujikawa, 1982; Iritani and Narita, 1984).

Significant dietary lipid effects are apparent with regard to the ratio of both monoenoic to polyenoic fatty acids and the n-6 to the n-3 series of polyunsaturated fatty acids in the membrane phospholipids, although the effects are often tissue-specific. Diets rich in the n-3 fatty acids elevate the proportions of 20:5 n-3, 22:5 n-3, and 22:6 n-3, often at the expense of 20:4 n-6 (Kurata and Privett, 1980; Holmer and Beare-Rogers, 1985). In addition, the level of dietary 18:2 n-6 influences the proportion of the docosenoic n-3 polyunsaturated fatty acids present in the membrane lipids (Kramer et al., 1982). This inverse relationship between the n-3 and n-6 fatty acid series suggests competition for incorporation into membrane phospholipids, particularly in the heart (Gudbjarnason and Oskarsdottir, 1977). The implications of changes in the n-6 to n-3 fatty acids have been discussed (Budowski and Crawford, 1985). However, both human (Galloway et al., 1985) and rat (Iritani and Narita, 1984) platelets behave differently from other tissues in that 20:4 n-6, particularly when associated with membrane phosphatidylinositol, is very resistant to replacement by polyunsaturated fatty acids of the n-3 series. The proportion of the n-3 series can also be elevated when feeding diets low in both the n-6 series (primarily 18:2 n-6) and the n-3 series, as is

**TABLE 7–1. Dietary Lipid Effects on Membrane Properties: Membrane Lipid Compositional Changes**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane lipid composition | Reference |
|---|---|---|---|
| Rat liver mics | SSO, CCO, ± CHL; 4 wk | Mb CHL increase compensated by changes in Mb FA | Garg et al., 1985b |
| Rat liver PL | Ethyl 18:2, ethyl 18:3, ± CHL; 8 wk I.P. | Mb CHL influenced Mb FA for diet n-6 and n-3 FAs | Huang et al., 1985c |
| Rat liver PL | Variable CHL + SFO; 8 wk | 20:4n-6 decreased by CHL; Δ5-desaturase inhibited by CHL (?) | Huang et al., 1985a |
| Rat liver total lipids | Separate PUFAs as esters; 8 wk | Dietary n-3 PUFA decreased 20:4n-6 | Castor et al., 1976 |
| Rat liver mics | CCO, CO, Menhaden oil, 100 wk | n-3 PUFA incorporated; type and amount of fat influenced Δ6-desaturase | Kurata and Privett, 1980 |
| Rat liver mito, mics, platelets | CO, FO, fat-free→fat-supplemented; 4wk | Mb FA changes within 6 days; limited replacement of 20:4n-6 in PC | Iritani and Narita, 1984 |
| Rat liver RBC, PL | SFO, HCCO, EPO ± CHL; 5 wk | CHL influenced n-6 PUFA metabolism; inhibited Δ6, Δ5, and Δ4 desaturation (?) | Huang et al., 1984 |
| Rat liver platelets, PL | n-9, n-6, n-3 FA; 5 wk | In platelets, all classes readily incorporated into Mb PL | Weiner and Sprecher, 1984 |
| Rat liver and heart PL | CO, FO, 2 wk | n-3 PUFA competes with 20:4 n-6 for *sn*-2 position of Mb PL | Iritani and Fujikawa, 1982 |
| Rat liver plasma Mb, intestinal mucosa, brain synaptosomes, PL | P/S diets of 2.0 and 0.25; 1 wk | Dietary FA changed Mb PL classes and PL-FA n-6/n-3 ratio | Clandinin et al., 1983 |
| Rat heart adipose tissue, PL | SKF, SSO; 72 wk | Adipose FAs reflect diet FA; SKF increased heart 22:6n-3 | Charnock et al., 1985c |
| Rat liver, heart, kidney, and brain mito, mics, RBC; PL | SKF, SSO; 20 wk | % SFA not altered; n-6:n-3 ratio increased by SSO; decreased by SKF | Gibson et al., 1984 |
| Rat heart PL | SSO, LEAR, HEAR; 12 wk ± treadmill exercise | PL class distribution altered; FA composition altered by diet and exercise | Rocquelin, et al., 1981 |
| Rat heart mito, PL | SBO, HEAR; 11 day crossover | PC:PE ratio altered; FA changes in PC and PE more rapid than CLP | Innis and Clandinin, 1981a |
| Rat heart mito, PL | SBO, LEAR, HEAR; 3 wk | PL class distribution altered; PC increased with HEAR | Innis and Clandinin, 1981b |
| Rat heart mito, PL; plasmalogens | Trielaidin, 8 wk | Replacement of SFA with 18:1 trans in plasmalogens | Wolff et al., 1985 |
| Rat heart PL | CLO; 12 and 36 wk | Inverse relationship between n-6 and n-3 PUFA; adaptation at 36 wk | Gudbjarnason and Oskarsdottir, 1977 |
| Rat heart PL | SBO, LEAR; 16 wk | No change in % PL classes; diet 18:2 n-6 influenced C22 PUFA | Kramer et al., 1982 |
| Rat heart PL | Menhaden oil 5% to 20%; 3 wk | 20:4n-6 replaced by n-3 PUFAs; effect not proportional to n-3 level | Swanson and Kinsella, 1986 |
| Rat heart PL | CO, SBO, LEAR, olive oil; 16 wk | % SFA and PL classes not changed; FA changes of PC and PE differ from CLP | Kramer, 1980 |
| Rat heart and liver mito, mics; PL | HEAR; 5 wk | Erucic acid may inhibit turnover of 18:0/20:4 PC species | Ishinaga et al., 1982 |
| Rat heart total lipids | LSO, FO; 6 wk | 22:6n-3 replaced 20:4 n-6 in PL | Holmer and Beare-Rogers, 1985 |
| Rat heart PL | Hydrogenated SBO, CO; 20 wk | n-3 C22 PUFAs accumulate even with low dietary 18:3n-3 | Egwin and Kummerow, 1972 |
| Rat heart and liver mito, mics; total lipid | SBO, SSO, LEAR, hydrogenated SBO, CLO; 3 wk | Maintenance of UI and % SFA; n-6/n-3 PUFAs change; incorporation of 18:1 trans erucic acid | Tahin et al., 1981 |

*(Continued)*

**TABLE 7–1. Dietary Lipid Effects on Membrane Properties: Membrane Lipid Compositional Changes (Continued)**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane lipid composition | Reference |
|---|---|---|---|
| Rat major tissues and adipose tissue; PL | SSO, LSO, EPA; 6 wk (gavage) | n-6; n-3 ratio altered in heart; 18:2n-6 marker for adipose tissue changes | Roshanai and Sanders, 1985 |
| Rat major tissues; PL | EPO, SFO, SBO; 11 wk | EPO little effect on n-6 PUFAs; n-6 PUFAs replaced by n-3 PUFAs | Hoy et al., 1983 |
| Rat kidney PL | SFO, LSO, CLO, HCCO; 4 wk | Diet n-3 PUFA and metabolites replaced 20:4; 18:2n-6 in PL reflect diet 18:2n-6 | Croft et al., 1984 |
| Rat brain mics, synaptosomal Mb PL | SBO, SSO, LEAR; 24 days | Diet fat altered PC and Mb CHL levels; PC directly proportional to Mb CHL | Foot et al., 1982 |
| Rat brain RBC; PE, and PC | High and low P/S and n-6:n-3 ratios; 8 days | PL-FA profiles more responsive to changes in n-6:n-3 ratio than P/S ratio | Carlson et al., 1986 |
| Rat RBC, PL | Beef fat + CO at 5 and 25%; 4 wk | Type of fat more important than level of fat | Moses and Craig, 1983 |
| Pig heart total lipids | CO, HEAR; 8 wk | Erucic acid and eicosenoic esters precursors for alkenyl ethers of plasmalogens | Kramer and Hulan, 1977 |
| Mouse liver adipose tissue; PL | HCCO, SFO, EPO (ob/ob) | n-3 PUFAs decreased in obese mice | Cunnane et al., 1985 |
| Mouse liver, heart, spleen, and thymus PL | CCO, SSO; 5 wk | CCO diet increased mono-FAs; SSO diet increased PUFAs | Burns et al., 1983 |
| Mouse B and T lymphocytes, PL | CO, olive oil, cocoa butter; 20 wk | B and T cells equally affected by diet; PUFAs replace monoenoic UFAs | Buttke et al., 1985 |
| Hamster liver, RBC, PL | SFO, EPO, LSO, ± CHL, ± EtOH; 7 wk | EtOH effects on Mb FA dependent on dietary PUFA and CHL | Cunnane et al., 1986 |
| Marmoset monkey liver, heart, kidney, and brain, mito PL; RBC, PL | SKF, SSO; 22 wk | % SFA unchanged; n-6:n-3 ratio increased by SSO, decreased by SKF | McMurchie et al., 1986 |
| Marmoset monkey heart PL | SKF, SSO; 22 wk | Nonuniform distribution of FAs in PLs; proportion of PLs not changed | Charnock et al., 1985a |
| Human RBC, PL | Different n-6/n-3 PUFA milk formulas, breast milk; 24 wk | PE and PC PUFAs highest with human breast milk feeding | Putman et al., 1982 |
| Human platelet PL | EPA; 4 wk | 20:5 n-3 replaced 20:4 n-6 in PC and PE; no effect on PI- and PS-FAs | Galloway et al., 1985 |
| Human adipose biopsy tissue TG, PC, PE | 7-day food record | PL more resistant to diet changes than TG; 18:2 correlated with dietary 18:2 and P/S | Field et al., 1985 |
| Human cheek cell; PL | Vegetarian, nonvegetarian | % SFA and 18:2n-6 reflect diet fat intake | McMurchie et al., 1984c |
| Human cheek cell; PL | High and low P/S diets; 6 wk crossover | 18:2n-6 increased with cross from low to high P/S diet fat | McMurchie et al., 1984b |

SBO, soybean oil; SFO, safflower seed oil; SSO, sunflower seed oil; CHL, cholesterol; CO, corn oil; CCO, coconut oil; SKF, sheep kidney fat; CLO, cod liver oil; HCCO, hydrogenated coconut oil; FO, fish oil; LSO, linseed oil; EPO, evening primrose oil; LEAR, low erucic acid rapeseed oil; HEAR, high erucic acid rapeseed oil; EPA, eicosapentaenoic acid; FA, fatty acid; UFA, unsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; C 22, docosenoic; P/S, polyunsaturated to saturated fatty acid ratio; UI, unsaturation index; PL, phospholipid; TG, triacylglycerol; CLP, cardiolipin; PI, phosphatidylinositol PS, phosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; Mb, membrane; mics, microsomes; mito, mitochondria; RBC, red blood cell; EtOH, ethanol;ob/ob, obese; wk, week; IP, intraperitoneal.

observed when feeding rats and marmosets a sheep kidney (perirenal) fat-supplemented diet (Gibson et al., 1984; Charnock et al., 1985a,c; McMurchie et al., 1986).

The fatty acid profile of the major membrane phospholipids, phosphatidylcholine and phosphatidylethanolamine, has been shown to be readily and rapidly modified by dietary lipids, with significant changes occurring within 2–3 days in rat heart mitochondria (Innis and Clandinin, 1981a). Furthermore, the membrane fatty acid profile remains fairly constant once a dietary-induced alteration has been achieved (Buttke et al., 1985). Some uncertainty still exists regarding whether only the type of dietary fat (Moses and Craig, 1983) or both the type and the amount of dietary fat are important in determining the membrane lipid composition (Kurata and Privett, 1980).

A number of uncommon fatty acids have been used in dietary lipid studies, particularly in relation to their potential cardiotoxicity. Dietary elaidic acid (18:1 trans) and erucic acid (22:1 cis) are incorporated into the membrane lipids of liver and heart organelles (Tahin et al., 1981). In addition to erucic acid, esters of eicosenoic acids can serve as precursors of the alkenyl ethers of rat heart plasmalogens (Kramer and Hulan, 1977). Elaidic acid competes with saturated fatty acids but not with oleic acid during plasmalogen biosynthesis. As a consequence of the similarity between the physical properties of saturated and trans unsaturated fatty acids, the exchange of elaidic acid with saturated fatty acids would not be expected to lead to large changes in membrane fluidity (Wolff et al., 1985). It could also be argued, as is discussed above under Membrane Fluidity, that substitution of one type of long-chain, polyunsaturated fatty acid for another, e.g., n-6 for n-3, would not dramatically influence membrane lipid fluidity particularly if the major exchange was between 20:4 n-6 and 22:6 n-3, as appears to be the case (Table 7–1). However, in the absence of molecular species analysis and biophysical determination of membrane fluidity following compositional changes, the effect of substituting different series of long-chain, polyunsaturated fatty acids on membrane fluidity remains uncertain. Changes in the ratio of the monoenoic to polyenoic fatty acids in the membrane lipids could potentially influence membrane fluidity because of the disproportionate contribution provided by the number of double bonds to membrane fluidity, as discussed previously (Stubbs et al., 1981; Coolbear et al., 1983). It could be argued that a relative increase in the number of monenoic fatty acids at the expense of the polyenoic fatty acids would tend to increase membrane fluidity. Therefore, some of the effects of increased monoenoic cis unsaturated fatty acids, such as oleic, gadoleic, erucic, and cetoleic acids, could be mediated by effects on membrane fluidity.

One issue that remains unresolved is the effect of dietary fatty acid supplementation on membrane phospholipid class distribution. Several studies have clearly shown that no change in phospholipid class distribution occurs in response to feeding diets of widely different fatty acid compositions (Kramer, 1980; Ishinaga et al., 1982; Charnock et al., 1985a,c; Swanson and Kinsella, 1986). On the other hand, the ratio of phosphatidylcholine to phosphatidylethanolamine has been reported to be altered in rat heart mitochondria after feeding diets of different fatty acid composition (Innis and Clandinin, 1981b). In addition, there are reports that other membranes, such as brain synaptosomal membranes (Foot et al., 1982), rat liver plasma membranes, and intestinal cell-surface membranes (Clandinin et al., 1983), display this trend. If changes in the distribution of membrane phospholipids do occur in response to dietary fatty acid supplementation, it is conceivable that changes in membrane fluidity also occur. This would be particularly so if the ratio of sphingomyelin to phosphatidylcholine or the ratio of phosphatidylethanolamine to phosphatidylcholine was altered; each of these phospholipids displays different physical and structural properties, which could influence

**TABLE 7–2. Dietary Lipid Effects on Membrane Properties: Biophysical Measurements**

| Animal/tissue membrane/biophysical measurement | Diet and duration | Major or inferred effect on membrane fluidity | Reference |
|---|---|---|---|
| Rabbit platelet Mb, DPH FP | CCO, CO, milk fat; 6 m | Fluidity directly correlated with Mb PUFA content | Berlin et al., 1980 |
| Human RBC PL; DPH FP, ESR SL | EPA, DHA; 8 wk | Changes in Mb FA and % PL classes maintain constant fluidity | Popp-Snijders et al., 1986 |
| Tumor cells in mice, Mb PL, ESR SL | Fat-free; 2 wk | Mb lipids from fat-free mice; less fluid, higher $T_c$ for $\tau_o$ | King et al., 1977 |
| Quail, RBC Mb, ESR SL; ± EtOH | ± CHL; 16 wk | Diet CHL increased S and C/PL; increased C/PL blocked disordering effect of EtOH | Chin and Goldstein, 1984 |
| Human ± diabetes; RBC Mb, ESR SL | Sardine oil (with EPA); 8 wk | At start, diabetic Mb less fluid than control; diet increased Mb EPA, decreased C/PL; Mb fluidity the same in each dietary group | Kamada et al., 1986 |
| Rat liver mics, total lipids; DSC | Fat-free diet; 1 wk | No phase transition in control, but evident after fat-free diet | Mabrey et al., 1977 |
| Rat liver and heart mito, lipids, DSC | SSO, SKF; 16 wk | Two phase transitions observed with all diets; transition temperature lowered with SSO, increased with SKF diet | McMurchie et al., 1983b |
| Rat liver mics, total lipids; DSC | CCO, SSO, ± CHL; 4 wk | Two phase transitions observed with all diets; transition temperature lowered with increased Mb CHL and SSO diet | Garg et al., 1985a |

DPH, 1,6-diphenyl-1,3,5-hexatriene; FP, fluorescence polarization; m, months; DHA, docosahexaenoic acid; ESR, electron spin resonance; SL, spin label; $T_c$, Arrhenius critical temperature; $\tau_o$, spin label motion; C/PL, cholesterol to phospholipid ratio; S, order parameter; T, temperature; DSC, differential scanning colorimetry. All other abbreviations as in Table 7–1.

membrane fluidity (see above under Membrane Fluidity).

Dietary cholesterol, with or without fatty acid supplementation, has been shown to influence membrane fatty acid composition (Garg et al., 1985a,b; McMurchie et al., 1987, 1988). This probably reflects the homeostatic capacity of the cell to restore membrane fluidity to original levels following perturbations by changing the levels of membrane cholesterol. It has also been inferred that dietary cholesterol inhibits Δ6- and Δ5-desaturase activity (Huang et al., 1984; 1985a,c), which could also explain the effect of cholesterol on membrane fatty acid composition. In studies in which lipid composition is the only parameter measured, the supposition that increased levels of membrane cholesterol will always lead to a decrease in membrane fluidity must be weighed against any homeostatic tendency of the cell to restore membrane fluidity by other mechanisms, such as those described above under Assembly and Compositional Variations of Membrane Phospholipids.

## Membrane Physical Properties and Fluidity

The effects of dietary lipids on membrane lipid fluidity are listed in Table 7–2. Membrane fluidity has been measured by several biophysical techniques, including the use of fluorescent and spin-labeled probes and differential scanning calorimetry, and a wide range of dietary lipid treatments has been used in these studies.

Many factors can influence membrane fluidity (see section on Membrane Fluidity

above), so it is not surprising that the relationship between dietary lipids, membrane lipids, and membrane fluidity is complex. Nevertheless, some trends are apparent. For example, the unsaturated to saturated fatty acid ratio of rabbit platelet plasma membrane lipids was shown to be inversely proportional to membrane microviscosity determined by 1,6-diphenyl-1,3,5-hexatriene (DPH) steady-state fluorescence polarization (Berlin et al., 1980), implying that the proportion of unsaturated membrane lipids was directly proportional to membrane fluidity. For rat lacrimal gland plasma membranes, an increase in the proportion of elaidic acid (18:1 trans) following dietary supplementation with partially hydrogenated soyabean oil decreased membrane fluidity as determined by the DPH fluorescent probe (Alam et al., 1985). An increase in lipid unsaturation of rat intestinal plasma membranes following dietary corn oil supplementation also increased membrane fluidity as determined by DPH fluorescence anisotropy (Brasitus et al., 1985). These results indicate that membrane lipid saturation/unsaturation influences membrane lipid fluidity. However, this apparent relationship is not observed in other studies, reinforcing the point that gross lipid analysis of membranes yields data insufficient to predict membrane fluidity accurately. This is supported by the study by Momchilova et al. (1985) in which no difference was detected in the fluidity of rat liver plasma membranes determined by DPH fluorescence from animals on control and sunflower seed oil-supplemented diets despite large differences in membrane fatty acid composition. Dietary changes in the ratio of both the monoenoic to polyenoic unsaturated fatty acids and the n-6 to the n-3 series of polyunsaturated fatty acids did not alter the fluidity of rat heart sarcolemmal and sarcoplasmic reticular membranes as determined by steady-state fluorescence polarization of DPH and polarization gradients for a series of *n*-(9-anthroyloxy) fatty acid probes (Abeywardena et al., 1984). Furthermore, shifts in the proportion of individual fatty acids leading to a 5% change in the proportion of unsaturated fatty acids of rat erythrocyte membrane phospholipids did not influence spin-label motion, supporting the interpretation that a constant membrane fluidity was maintained despite changes in lipid unsaturation (Ehrström et al., 1981).

Whereas membrane fatty acid composition may play an important role in determining membrane fluidity, this effect may be counteracted by changes in other membrane lipid components. For example, although changes in the lipid composition of rat liver plasma membranes induced by a starve–refeed–starve regimen were shown to increase the double bond index and to induce a reduction in membrane lipid saturation, the increased membrane fluidity determined by both fluorescence polarization of DPH and 12-anthroyloxy stearate, as well as by intramolecular excimer fluorescence of the probe DPP (1,3-di[1-pyrenyl]propane), may have been the result of a decreased cholesterol to phospholipid (C/PL) ratio occurring in concert with the changes in membrane lipid saturation (Storch and Schachter, 1984). In this regard, the membrane C/PL ratio may be more directly related to membrane fluidity than the multitude of membrane fatty acid changes that can result from dietary lipid supplementation. For example, an increase in the C/PL ratio of quail erythrocyte membranes following dietary cholesterol supplementation decreased membrane fluidity as determined by spin-labeling techniques (Chin and Goldstein, 1984; Lurie et al., 1985). A decrease in the fluidity of guinea pig liver microsomal membranes determined by fluorescent probe techniques was also observed following dietary cholesterol supplementation (Castuma and Brenner, 1986). In this latter study, the increased membrane C/PL ratio was accompanied by a decreased phosphatidylethanolamine/phosphatidylcholine ratio, which, in some circumstances, could counteract the influence of a change in the membrane C/PL ratio.

The interaction between different types of membrane lipids in determining membrane fluidity was shown for human erythrocyte

membranes by Popp-Snijders et al. (1986). Although the erythrocyte membrane fatty acid composition and unsaturation index were altered by dietary lipid supplementation, membrane fluidity determined by spin-labeling techniques was unaltered because of a compensatory change in the phosphatidylethanolamine/phosphatidylcholine ratio (Popp-Snijders et al., 1986). In addition to changes solely in the membrane lipid components, dietary lipids may induce changes in the membrane phospholipid to protein ratio, which could significantly influence membrane fluidity, as discussed above under Membrane Fluidity. Indeed, an increase in the phospholipid to protein ratio of rabbit platelet plasma membranes following dietary lipid supplementation has been shown to be directly proportional to membrane fluidity as determined by DPH fluorescence polarization (Berlin et al., 1984). We have also observed, with rat heart sarcolemmal membranes, that, in addition to an increase in the membrane C/PL ratio, dietary cholesterol supplementation also decreases the membrane (total) lipid to protein ratio (McMurchie et al., 1987).

Temperature has been used in conjunction with both probe and nonprobe techniques to compare membrane fluidity following some form of dietary perturbation. The rationale for the use of temperature relates to the fact that the disorder⇌order phase transition of the membrane lipids, which is determined primarily by the membrane lipid composition (McElhaney, 1982), reflects some aspect of the lipid–lipid interactions within the membrane bilayer. As well as being used to determine the thermotropic behavior of membrane lipids, differential scanning calorimetry (DSC) has also been used to compare the relative fluidity of membranes following the dietary-induced changes in lipid composition. Two distinct lipid phase transitions have been detected in membranes and extracted lipids of rat liver and heart mitochondria (McMurchie et al., 1983b), rat liver microsomes (Garg et al., 1985a), and beef heart submitochondrial particles (Blazyk and Newman, 1980). In these studies, the exothermic phase transition occuring at the higher temperature (beginning between 24°C and 14°C), exhibited a low enthalpy value compared with the broad exothermic phase transition beginning at about 0°C. The lower-temperature transition, which continued down to temperatures well below 0°C, probably represents the phase transition of the majority of the (poly)unsaturated membrane lipids. The higher-temperature, lower-enthalpy transition may result from a domain of disaturated phospholipids that undergoes some form of transition independent of the main phase transition (McMurchie et al., 1983b).

Dietary modification of membrane lipids influences the transition temperature of the lower-temperature phase transition. For rat liver mitochondria, dietary sunflower seed oil decreased the temperature of this phase transition, whereas sheep kidney fat had the opposite effect (McMurchie et al., 1983b). A similar result was observed with rat liver microsomal membrane lipids following dietary sunflower seed oil or coconut oil supplementation (Garg et al., 1985a). In that the temperature of this phase transition probably reflects the relative fluidity of the majority of the mitochondrial membrane lipids, an increase in the transition temperature (as occurred when feeding a predominantly saturated fatty acid diet) would indicate a decrease in membrane fluidity, with the opposite being produced by dietary unsaturated fatty acids. The failure to observe dietary effects on the higher-temperature, lower-enthalpy transition implies that the lipids involved in this transition are not readily influenced by dietary lipids or by changes in the fluidity of the majority of the membrane lipids (McMurchie et al., 1983b). An increase in membrane cholesterol following dietary cholesterol supplementation decreased the phase transition temperature of rat liver microsomal membrane lipids irrespective of the nature of the accompanying dietary fatty acid supplement (Garg et al., 1985a). This result contrasts with the reported effect of cholesterol on membrane fluidity (Quinn, 1981; McElhaney, 1982) but may be explica-

**TABLE 7–3. Dietary Lipid Effects on Membrane Properties: Biophysical and Functional Measurements**

| Animal/tissue membrane/biophysical measurement | Diet and duration | Major or inferred effect on membrane fluidity | Reference |
|---|---|---|---|
| Rabbit liver mito, ESR FL | Fat-free, CO, HCO, HCCO; 28 days | Slower oscillation frequency and spin-label motion with EFA-deficient rats | Williams et al., 1972 |
| Rat, sheep; liver and kidney mito; ESR SL | SSO, variable | $E_a$ and $T_c$ of succinate oxidation inversely proportional; $T_c$ decrease with SSO | McMurchie and Raison, 1979 |
| Rat heart mito, SM, SR; FP-AS, DPH FP | SSO, SKF; 9 wk | No effect on $E_a$ or $T_f$ of $F_1$-ATPase, NaK-ATPase, Ca-ATPase | Abeywardena et al., 1984 |
| Rat intestinal Mb; DPH vs.T | Butterfat, CO; 6 wk | CO diet reduced Mb fluidity and increased NaK-ATPase | Brasitus et al., 1985 |
| Rat liver plasma Mb, DPH | SSO, HSSO; 10 wk | HCCO decreased fluidity; SSO increased 5′nucleotidase; HSSO decreased P'lipase $A_2$ | Momchilova et al., 1985 |
| Rat liver aorta, mics; DPH FL | SFO, fat-free; 15 wk | Mb fluidity not altered; SFO decreased Δ6-desaturase | Holloway and Holloway, 1977 |
| Rat liver plasma Mb; DPH FP, FP-AS | Starve ⇄ refeed regimen | C/PL decreased; Mb fluidity increased; NaK-ATPase increased | Storch and Schachter, 1984 |
| Quail RBC Mb; ESR SL | ± CHL; 16 wk | C/PL increased; Mb fluidity and AC activity decreased; BAR unaffected | Lurie et al., 1985 |
| Rat liver plasma Mb; ESR SL | High CHL, CHL + Clofibrate; 4 wk | Low C/PL Mb, higher AC activity, lower stim. by agonists | Needham et al., 1985 |
| Rat lacrimal gland plasma Mb; DPH FP | CO, PHSBO; 24 wk | PHSBO decreased Mb fluidity, increased AC activity | Alam et al., 1985 |
| Guinea pig liver mics, DPH FP | CHL; 25 days | C/PL increased; PE/PC decreased; Mb fluidity decreased; UDP-glucuronyl transferase activity increased | Castuma and Brenner, 1986 |
| Rat RBC, Mb, ESR SL | High EFA, low EFA | No change in Mb fluidity; low EFA increased sensitivity to osmotic lysis | Ehrström et al., 1981 |
| Rabbit platelet Mb; DPH FP | CO, CCO, milk fat, cocoa butter, 200 and 400 days | Age-related changes in PL: protein and Mb fluidity; diet effects not great | Berlin et al., 1984 |

EFA, essential fatty acid; HCO, hydrogenated corn oil; $E_a$, Arrhenius activation energy; SM, sarcolemmal membrane; SR, sarcoplasmic reticulum membrane; ATPase, adenosine triphosphatase; FP-AS, fluorescent probe, anthroyloxy stearate; HSSO, hydrogenated sunflower seed oil; AC, adenylyl cyclase; BAR, β-adrenergic receptor; PHSBO, partially hydrogenated soybean oil. All other abbreviations as in Tables 7–1 and 7–2.

ble in terms of some form of homeostatic change in the membrane fatty acid composition following increased incorporation of cholesterol into the microsomal membrane (Garg et al., 1985a). The DSC studies of Mabrey et al. (1977) differ from those cited above in that no phase transition was detected above 0°C for rat liver microsomes until rats were fed a fat-free diet. Factors such as instrument sensitivity, scan rate, and sample preparation might partly explain some of these differences.

The data presented in Table 7–3 summarize dietary-lipid studies in which both membrane fluidity and some aspect of membrane function have been determined in the same study. It is clear that considerable variation in the behavior of membrane-associated enzymes

occurs in response to changes in membrane fluidity. For example, although the oscillation period and freedom of spin-label motion were significantly altered in rat liver mitochondria by diet-induced changes in the membrane unsaturated fatty acid composition, ion permeability and oxidative phosphorylation were not affected (Williams et al., 1972). However, other studies, which will be discussed in reference to Table 7–4, show an effect of dietary lipids on mitochondrial membrane function, including oxidative phosphorylation.

The activity of rat intestinal plasma membrane-associated $(Na^+,K^+)$-ATPase was shown to increase with increasing membrane fluidity, although the activity of ρ-nitrophenylphosphatase was unaffected (Brasitus et al., 1985). Furthermore, the activities of 5′-nucleotidase and phospholipase $A_2$ associated with rat liver plasma membranes were shown to change in opposite directions for similar changes in membrane fluidity (Momchilova et al., 1985). The activity of $(Na^+, K^+)$-ATPase was increased when the fluidity of rat hepatocyte plasma membranes was increased by a starve–refeed–starve regimen (Storch and Schachter, 1984). A decrease in the fluidity of guinea pig liver microsomal membranes induced by an increased membrane C/PL ratio increased the $V_{max}$ and the Hill coefficient and decreased the $k_m$ for the membrane enzyme UDP-glucuronyl transferase (Castuma and Brenner, 1986). These results indicate apparent differences in the extent to which different enzyme proteins are associated with the membrane, the lipid domain(s) within which enzyme proteins reside, or the nature of the lipid–protein interactions dictating aspects of their behavior. Under other circumstances, the activity of some membrane enzymes may be unrelated to changes in the membrane fluidity. For example, no correlation was found between Δ9-desaturase activity (measured as stearoyl-CoA desaturase) and the fluidity of rat liver or aortic microsomal membranes determined by DPH fluorescent probe techniques following a fat-free or safflower oil diet (Holloway and Holloway, 1977).

The activity of membrane-associated adenylyl cyclase(s) appears to be particularly sensitive to membrane lipid fluidity. However, this enzyme-receptor system has been reported to exhibit both a direct and an inverse relationship to membrane fluidity depending on the particular study. Thus adenylyl cyclase activity decreased when membrane fluidity was reduced following an increase in the membrane C/PL ratio (Lurie et al., 1985). However, adenylyl cyclase activity associated with rat lacrimal gland plasma membranes was shown to increase when the fluidity was decreased by feeding a partially hydrogenated soyabean oil supplement (Alam et al., 1985). In the study of Needham et al. (1985), the response of glucagon-stimulated adenylyl cyclase associated with rat liver plasma membranes to changes in membrane fluidity induced by an alteration in the membrane C/PL ratio was complex. Higher rates of basal and sodium flouride (NaF)-, forskolin-, and glucagon-stimulated adenylyl cyclase activity were apparent in the more fluid membranes (low C/PL ratio and order parameter), but the extent to which these agonists stimulated adenylyl cyclase activity was greatest in the less fluid membranes (high C/PL ratio and order parameter). For rat heart membrane-associated, catecholamine-stimulated adenylyl cyclase activity, we have observed a significant increase in activity following an increase in the membrane C/PL ratio induced by dietary cholesterol (McMurchie et al., 1987). This result agrees with studies of Sinensky et al. (1979) demonstrating that increased membrane fatty acyl chain ordering activates adenylyl cyclase. However, although many studies show that (hormone-sensitive) adenylyl cyclases are sensitive to changes in membrane lipid fluidity, an explanation of their response to altered membrane fluidity is probably related to a consideration of the optimum level of membrane fluidity required to activate this enzyme complex. The departure from some optimal fluidity level, as a result of an increase or a decrease in membrane fluidity, may change the activity of this enzyme complex and/or its sensitivity to var-

ious agonists. Data showing this biphasic response of adenylyl cyclase to membrane fluidity have been presented by Houslay (1985). It is conceivable that other membrane enzymes also show a biphasic response to altered membrane fluidity, reinforcing the possibility that membrane fluidity, within any one membrane, is not identical in those regions of the membrane associated with different enzyme proteins. This may provide each of the membrane enzymes with a unique set of fluidity conditions, which allow that enzyme to function at its most optimal level. At present, methods for the measurement of membrane fluidity in intact membranes cannot accurately discriminate the lipid microenvironment around a particular membrane enzyme complex and thus report on its physical properties.

Arrhenius plots of membrane-associated enzyme activity have also been used (often in conjunction with spin-labeling techniques) to investigate the relationship between diet-induced alterations in membrane fluidity and membrane enzyme function. McMurchie and Raison (1979) reported that supplementation of rat and sheep diets with sunflower seed oil (casein-protected for sheep) lowered the characteristic transition temperature for the Arrhenius plot of spin-label motion in liver and kidney mitochondria. This characteristic temperature for spin-label motion also corresponded with the characteristic "break" temperature evident in Arrhenius plots of mitochondrial succinate oxidase activity. These data were taken as evidence that the altered mitochondrial membrane lipid composition induced by dietary sunflower seed oil increased the fluidity of the membrane lipid domain probed by the spin label. This study also demonstrated a unique relationship between the Arrhenius activation energy for membrane-associated enzyme activity and the parameter $T_c$, i.e., the critical or "break" temperature derived from Arrhenius plots of spin-label motion and membrane enzyme activity (McMurchie and Raison, 1979). This relationship, which has been confirmed in more recent dietary studies (McMurchie et al., 1983a,c), implies that the activation energy of this particular (multicomponent) membrane-enzyme system is directly proportional to the fluidity of the inner mitochondrial membrane. Even when the discontinuity in the Arrhenius plot was abolished by the addition of low concentrations of nonionic detergents, the activation energy for succinate oxidase still reflected this relationship to membrane lipid fluidity (McMurchie and Raison, 1979).

In other studies, a reduction in the C/PL ratio of rat liver plasma membranes by dietary clofibrate treatment abolished the higher characteristic temperature for spin-label motion, which was considered indicative of dietary-induced differences in the transmembrane distribution of cholesterol (and hence lipid fluidity) between inner and outer halves of the membrane bilayer (Needham et al., 1985). In that study, Arrhenius plots of hormone-sensitive adenylyl cyclase activity displayed different profiles depending on the membrane C/PL ratio. The study by King et al. (1977) reported that one of the two characteristic Arrhenius transition temperatures for the motion of spin labels in tumor cell membranes grown in a mouse host increased in temperature when mice were fed a fat-deficient diet. This indicated a decrease in membrane fluidity, which was consistent with the observed membrane lipid changes.

Diet-induced changes in membrane-lipid composition have not always been associated with changes in the profile of Arrhenius plots of membrane enzyme activity, possibly for some of the reasons discussed above under Lipid Modulation of Membrane Proteins. For example, in the study by Brasitus et al. (1985), no effect of dietary lipid supplementation on the Arrhenius plot of ρ-nitrophenylphosphatase activity was detected despite significant changes in membrane fluidity determined by DPH fluorescent probe techniques. In the study by Abeywardena et al. (1984), Arrhenius plots of rat heart sarcolemmal membrane $(Na^+,K^+)$-ATPase and sarcoplasmic reticulum $Ca^{2+}$-ATPase activities were not significantly different following alterations in the lipid composition of

the respective membranes induced by dietary sunflower seed oil or sheep kidney fat supplementation. Finally, in the study by McMurchie et al. (1983a), employing dietary supplementation identical to that of Abeywardena et al. (1984), Arrhenius plots of rat heart mitochondrial $F_1$-ATPase activity were unaltered by the dietary lipid treatment (as was the case for the ATPases of the sarcolemmal and sarcoplasmic reticulum membranes), although significant alterations in the Arrhenius plots for succinate cytochrome c reductase activity were apparent. For rat heart mitochondrial succinate cytochrome c reductase activity, the changes in the profiles of the Arrhenius plots (McMurchie et al., 1983a) again indicated a direct relationship between the Arrhenius activation energy ($E_a$) and the apparent membrane fluidity, as was first evident in the study by McMurchie and Raison (1979). Differences in the thermotropic behavior of various membrane ATPases and the mitochondrial electron transport chain in membranes of differing lipid compositions and fluidities may be attributable to the multicomponent nature of the electron transport chain and the need for mobility of its intrinsic components in the lateral plane of the membrane as a corequisite for function (Hackenbrock et al., 1976). This molecular behavior of the mitochondrial electron transport chain, contrasting with that of the ATPases, which may be in a relatively static orientation in the membrane for functional purposes, is comparable to the situation for hormone-sensitive adenylyl cyclases, where mobile receptors and collision coupling are believed to occur (Houslay, 1981; Helmreich and Elson, 1984). Therefore, it may well be that those multicomponent membrane enzyme systems requiring movement of some or all of their components in the lateral plane of the membrane for expression of activity, are the membrane enzymes most susceptible to diet-induced alterations in membrane lipid fluidity.

The studies described above suggest that lipid fluidity can influence membrane enzyme function in several ways. Membrane enzyme activity (or some parameter thereof) may be directly or inversely proportional to changes in membrane lipid fluidity, or, alternatively, there may be no correlation between the two. A lack of correlation may apply only as long as the membrane lipids are in a liquid-crystalline (fluidus) phase. The formation of "solidus" phase lipid within the membrane, which can occur at low temperatures, may be sufficient to influence the function of those enzymes that would not normally respond to increases or decreases in membrane fluidity while the membrane lipids remained in a fluid phase configuration. A particular membrane enzyme may also show a biphasic response to fluidity changes as a reflection of the optimal level of membrane fluidity conducive to maximal functional efficiency. Finally, diet-induced changes in membrane fluidity may influence parameters other than enzyme activity, such as platelet aggregation. Although this is covered in the next section, platelet aggregation was reported to be directly proportional to platelet plasma membrane fluidity measured by DPH fluorescent probe techniques (Berlin et al., 1984).

### Membrane-Associated Functional Activity

A summary of the effects of dietary lipids on various cellular processes is given in Table 7–4. Although this summary is by no means comprehensive, it is clear that dietary lipids manifest their effects at different levels and on many different cellular functions. Many of the studies listed in Table 7–4 also report significant changes in membrane lipid composition, which were similar to the changes discussed in relation to Tables 7–1, 7–2, and 7–3. The major implication of the data in Table 7–4 is that dietary lipids exert their effects on functional activity via changes in membrane physicochemical properties.

The role of dietary lipids in regulating the activity of membrane-associated desaturases points to the possibilities that the desaturase enzymes themselves are sensitive to membrane fluidity and, in some circumstances, contribute to self-regulation of the fluidity of

**TABLE 7–4. Dietary Lipid Effects on Membrane Properties: Membrane-Associated Functional Changes**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane function | Reference |
|---|---|---|---|
| Membrane desaturase and lipid metabolizing enzymes | | | |
| Rat liver mics; platelets | PH vegetable oils, PH FO, t-FAs; 10 wk | Δ5, Δ6-Desaturase increased with low 18:2n-6 diets | Blomstrand et al., 1985 |
| Rat liver mics | PH CCO, HEAR, PH FO; t-FAs; 10 wk | Δ5, Δ6-Desaturase activity decreased with PH FO | Svensson, 1983 |
| Rat liver and heart Mb | PH PO, RSO, 18:1,22:1, c and t isomers | Δ6-Desaturase activity decreased with t monoenoic diet | Holmer et al., 1982 |
| Rat (SHR) liver mics | CO, CCO; 6 to 16 wk | Δ9, Δ6, Δ5-Desaturase increased with CO, decreased with CCO; no effect on ACAT | Pugh and Kates, 1984 |
| Rat liver mics | Tristearin, SFO, ± CHL; 12 h | HMG-CoA reductase and ACAT activity affected in opposite directions by diets | Mitropoulos et al., 1980 |
| Rat liver mics | 18:3n-6, 18:2 t,t, 16:0, 20:4n-6, ethyl esters; 12 to 48 hr | Δ5-Desaturase increased via enzyme synthesis; Δ6 and Δ5-desaturase affected in opposite directions | de Gomez Dumm et al., 1983 |
| Rat liver mics | HCCO, FO, SFO, EFA-deficient diet; 33 wk | Diet n-3 PUFAs increase minimum 18:2n-6 requirement | de Schrijver and Privett, 1982 |
| Mitrochondrial respiration and associated enzyme activities | | | |
| Rat liver mito | Beef tallow, CO; 3 wk | CO diet increased Cyt c oxidase activity and lowered $T_c$ | Abuirmeileh and Elson, 1980a |
| Rat liver mito | Beef tallow, SFO; 4 wk | SFO diet increased Cyt c oxidase, decreases ATP/Pi exchange; Succ oxid not affected | Abuirmeileh and Elson, 1980b |
| Rat liver mito | Beef tallow, SFO; 5 wk | SFO diet increased St 3 and St 4 Succ oxid and $K_m$ for ADP in ADNT reaction | Mak et al., 1983 |
| Rat liver, heart, skeletal muscle | EFA-deficient diet; 30 wk | Respiratory activities increased only in liver mito | Rafael et al., 1984 |
| Rat liver mito | EFA-deficient, CO; 9 wk; then 3 days PUFAs | EFA-deficient diet decreased mito oscillation frequency; restored with EFA supplementation | Williams et al., 1975 |
| Rat (± thyroid) liver, mito | CO, HCCO, 20:4n-6 (AR-diet); 10 wk | AR diet increased BHB-DH and Cyt c oxidase activities; hypophysectomy abolished dietary effect | Haeffner and Privett, 1975 |
| Rat heart mito | CO, SFO lard, HSBO; 6 wk | No effect on mito respiration despite Mb FA changed; adaptation (?) | Royce and Holmes, 1984 |
| Rat heart mito | PO, PH PO, RSO, PH FO; c and t isomers of 18:1 20:1, 22:1; 10 wk | PH FO and RSO inhibit St 3 respiration; 22:1 c inhibits ATP synthesis | Blomstrand and Svensson, 1983 |
| Rat heart mito | SBO, LEAR, HEAR; 16 wk | Substrate-specific diet effects; ADP/O (Succ) reduced with RSO diets; adaptation to long-term HEAR diet | Clandinin, 1978 |
| Rat, monkey (*Macaca* sp.) heart mito | HFO (high 22:11 c and t isomers); acute and chronic | Acute; decreased palmitoyl CoA oxidation; Chronic; adaptation to C22 FAs; monkey metabolizes C22 FAs rapidly | Forsyth et al., 1977 |
| Rat, marmoset (*Callithrix* sp.) liver mito | SKF, SSO; rat, 16 wk, marmoset, 22 wk | Substrate oxidation decreased in marmoset with high-fat diets; less so in rat | McMurchie et al., 1984a |
| Rat heart mito | SKF, SSO; 9 wk | SCRase activity unaffected; $T_c$ increased with SKF; $F_1$-ATPase activity increased with SKF; $T_c$ unaffected | McMurchie et al., 1983a |

*(Continued)*

**TABLE 7–4. Dietary Lipid Effects on Membrane Properties: Membrane-Associated Functional Changes (Continued)**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane function | Reference |
|---|---|---|---|
| Mitrochondrial respiration and associated enzyme activities (continued) | | | |
| Rat liver mito | SKF, SSO; 9 wk | SCRase activity unaffected; $T_c$ increased with SKF diet | McMurchie et al., 1983c |
| Rat heart mito | High and low P/S diets, with high and low % fat; 2 wk | $F_1$-ATPase activity increased with low fat and with low P/S diet | Robblee and Clandinin, 1984 |
| Rat heart mito | SBO, HEAR, long-chain FAs, short-chain FAs; 12 days | Long-chain FA diets increase Oligo sensitivity and DNP stimulation of $F_1$-ATPase | Zsigmond and Clandinin, 1986 |
| Rat heart mito | SBO, RSO; crossover 11 days | RSO diet decreased ATP/Pi exchange; rapid, reversible changes in ATPase after crossover | Innis and Clandinin, 1981c |
| Chicken heart mito | SSO, HEAR, LEAR; 24 days | Both RSO diets reduce ADP/O ratio and ATP synthesis | Renner et al., 1979 |
| General metabolic effects | | | |
| Rat liver plasma Mb | ± EFA diet; 6 wk | ATPase activity increased; 5′nucleotidase and hormone-stim. AC activity decreased with EFA-deficient diet | Brivio-Haughland et al., 1976 |
| Rat liver plasma Mb | High fat (lard); 5 days | 50% reduction in insulin binding sites | Sun et al., 1977 |
| Rat liver plasma Mb | LEAR, SFO, SBO ± 18:1c mixes; 24 days | Diet fat altered glucagon-stim. AC activity; dependent on level of diet LEAR | Neelands and Clandinin, 1983 |
| Rat liver plasma Mb | Low and high 18:2n-6; 24 days | Low 18:2n-6 diet increased glucagon and NaF-stimulated AC activity | Morson and Clandinin, 1985 |
| Guinea pig liver plasma Mb | ± CHL, ± ascorbic acid; 9 wk | Increased Mb C/PL decreased $Ca^{2+}$ uptake and CaATPase activity | Deliconstantinos et al., 1983 |
| Mouse liver nuclear envelope | High and low P/S diets; 4 wk | Low P/S diet reduced NTPase activity and T3 binding | Venkatraman et al., 1986 |
| Rat epididymal fat pads | SFO, CCO; 4 wk | SFO diet increased basal and NE-stimulated lipolysis and C/PL ratio | Awad and Zepp, 1979 |
| Rat epididymal fat pads, plasma Mb | SFO, CCO, beef fat; 4 wk | SFA diet reduced Mb C/PL, $K_d$, and $B_{max}$ for DHA binding to BAR | Awad, 1983 |
| Rat adipocytes | CCO, beef fat, SFO; 4 wk | CCO increased basal FFA levels; hormone-sensitive lipase unaffected by diet fat | Awad and Chattopadhyay, 1986 |
| Rat epididymal fat pads, Mb ghosts | CCO, beef fat, SFO; 4 wk | SFO diet increased NaF, Isop, NE stim., AC activity, and cAMP content of adipocytes | Awad and Chattopadhyay, 1986b |
| Rat epididymal fat pads, adipocytes | SSO, palm oil; 7 wk | SSO diet increased $B_{max}$ of low-affinity insulin binding sites | van Amelsvoort et al., 1986 |
| Cardiac enzymes and cardiovascular effects | | | |
| Rat heart atria; homog mics | CCO, SSO; 14 wk | CCO increased NE- and NaF stim. AC activity; SSO decreased $K_d$ and $B_{max}$ for DHA binding to BAR | Wince and Rutledge, 1981 |
| Rat heart SM | SFO, CCO; 4 wk | CCO decreased PDEase, pNPPase, and 5′nucleotidase activity | Awad and Chattopadhyay, 1983 |
| Rat heart SM | SKF, SSO, ± CHL; 20 wk | SKF and SSO no effefct on BAR or AC activity; CHL increased catecholamine-stim. AC activity; BAR down-regulated; correlated with Mb C/PL ratio | McMurchie, 1986; McMurchie, et al., 1987 |

*(Continued)*

**TABLE 7–4. Dietary Lipid Effects on Membrane Properties: Membrane-Associated Functional Changes (Continued)**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane function | Reference |
|---|---|---|---|
| Cardiac enzymes and cardiovascular effects (continued) | | | |
| Marmoset (*Callithrix* sp.) heart, SM | SKF, SSO; 80 wk | SKF increased catecholamine-stim. AC activity; BAR down-regulated; correlated with Mb C/PL ratio | McMurchie, 1986; McMurchie, et al., 1988 |
| Pig heart SR | Atherogenic diet (+ CHL); 55 wk | Ca ion uptake uncoupled from SR-ATPase activity; correlated with Mb C/PL ratio | Jacobson et al., 1985 |
| Rat heart; papillary muscle and perfused | EFA-deficient, 24 wk; SSO, 6 days | EFA-deficient diet decreased force of contraction; SSO increased coronary flow and ventricular work | Ten Hoor et al., 1973 |
| Rat heart; IP-Isop, mortality | CLO, ± vitamin E; 12 wk | Mortality from Isop stim., increased by CLO, decreased by vitamin E; correlated with increased Mb n-3 PUFAs | Gudbjarnason et al., 1978 |
| Rabbit heart, papillary muscle | Atherogenic diet (lard + CHL); 8 to 32 wk | Reduced cardiac contractility; Ca ion handling altered by Mb fluidity changes? | Peterson et al., 1979 |
| Rat, perfused heart | SSO, lard; 1 to 6 wk | SSO increased coronary flow rate and ventricular work; effect via NaK-ATPase? | dc Deckere and Ten Hoor, 1980 |
| Rat heart, papillary muscle | SSO, SKF; 12 to 45 wk | SKF increased inotropic response to Ca ions; SSO protected against Isop-induced arrhythmias | Charnock et al., 1985b |
| Rat heart contractility | SSO, various times | Diet 18:2n-6 reduced chronotropic and inotropic effects of NE and Isop | Hoffman, 1986 |
| Erythrocyte and platelet membrane functions | | | |
| Rat RBC Mb | EFA-deficient, CO, LSO, olive oil, lard; 15 wk | Mb fluidity directly proportional to H for $F^-$ inhib. of ACEase; inversely proportional to H for $F^-$ inhib. of NaK-ATPase | Bloj et al., 1973b |
| Rat RBC Mb | CO ± CHL; 1 to 4 wk | CHL decreased H for $F^-$ inhib. of ACEase; increased H for $F^-$ inhib. of NaK-ATPase | Bloj et al., 1973a |
| Rat RBC Mb; H values for ACEase ($F^-$), Ca-ATPase ($Ca^{2+}$) | CO, lard, hydrogenated fat; ± CHL; 15 wk | H value (cooperativity) altered by diet CHL when Mb fluidity high, i.e., with CO diet | Bloj et al., 1976 |
| Rat RBC Mb; insulin | CO, lard (variable time) | Insulin decreased H for $F^-$ inhib. of ACEase with CO diet; high initial Mb fluidity | de Melián et al., 1978 |
| Rat RBC Mb | CO, SFO, lard, HSBO; 6 wk | SFO increased RBC fragility by increased Mb 18:1c | Benga et al., 1984 |
| Human RBC Mb | 20:5n-3 and 22:6n-3 diet; 3 wk | Increased RBC deformability; reduced whole blood viscosity | Cartwright et al., 1985 |
| Human RBC platelets | 20:5n-3 ethyl ester; 4 wk | Platelet aggregation and whole blood viscosity decreased; RBC deformability increased | Terano et al., 1983 |
| Rat (SHR) paltelets | High SFA and PUFA diets; 16 wk | SFA increased platelet reactivity of normotensive and SHR rats | McGregor et al., 1981 |
| Rabbit platelets | CCO, LSO, FO, CCO; 9 wk | n-3 PUFAs decreased platelet aggregation to thrombin and collagen | Vas Dias et al., 1982 |

*(Continued)*

**TABLE 7–4. Dietary Lipid Effects on Membrane Properties: Membrane-Associated Functional Changes (Continued)**

| Animal/tissue membrane | Diet and duration | Major or inferred effect on membrane function | Reference |
|---|---|---|---|
| Miscellaneous effects | | | |
| Rat brain synaptosomal Mb | SBO, SSO, SBO PC; 4 wk | SSO increased ACEase activity; $T_c$ and $E_a$ altered | Foot et al., 1983 |
| Rat salivary gland, homog; kidney, homog | HCCO, CO, SFO, butter; 9 and 49 wk | HCCO and butter diets increased NaK-ATPase activity; inverse relationship with double bond index | Alam and Alam, 1983 |
| Rat peritoneal macrophages | CO, LSO; 4 wk | CO diet increased $B_{max}$ for prostaglandin $E_2$ areceptors and sensitivity of AC to stim. by $PGE_2$ | Opmeer et al., 1984 |
| Rat small intestinal brush border Mb | High and low 18:2n-6 diets; 17 days | Diet fat influenced active and passive transport of solutes in a selective manner | Thomson et al., 1986 |
| Rat heart brain Mb; EtOH tolerance, righting reflex | CCO, FO (continuous) | CCO increased tolerance to EtOH effects; correlated with increased SFA in Mb PL; Mb adaptation to EtOH | John et al., 1980 |
| Rat brain; sensitivity to anesthetics; total PL | EFA-deficient, 18:3n-3 ± 18:2n-6; 24 wk | EFA-deficient diet increased sensitivity to volatile anesthetics; supplementation with 18:2n-6 decreased sensitivity; correlated with 20:4n-6 content of PI | Evers et al., 1986 |

PO, peanut oil; RSO, rapeseed oil; PH, partially hydrogenated; HSBO, hydrogenated soybean oil; AR, arachidonic rich; ACAT, acyl-CoAa:sn-glycero-3-phosphocholine acyltransferase; ADNT, adenine nucleotide translocase; BHB-DH, β-hydroxybutyrate dehydrogenase; SCRase, succinate cytochrome c reductase; NTPase, nucleoside triphosphatase; FFA, free fatty acid; PDEase, phosphodiesterase I; pNPPase, p-nitrophenylphosphatase; ACE, acetylcholinesterase; c, cis; t, trans; H, Hill coefficient for cooperativity; Oligo, oligomycin; Cyt, cytochrome; DNP, 2,4-dinitrophenol; Succ, succinate; St, respiratory state; NE, norepinephrine; NaF, sodium fluoride; $F^-$, fluoride ion; DHA, $^3$H-dihydroalprenolol; $K_d$, dissociation constant; $B_{max}$, receptor number; Isop, isoproterenol; cAMP, 3,5-cyclic adenosine monophosphate; SHR, spontaneously hypertensive rat; homog, homogenate. All other abbreviations as in Tables 7—1 to 7–3.

their host membrane. However, verification of this fact awaits comparative studies on desaturase activity and membrane fluidity determined by appropriate biophysical techniques. Such a study should also include a direct assay of desaturase activity rather than assuming from membrane lipid analysis that the accumulation of a particular substrate(s) is always the result of an inhibition of desaturase activity. Regulation of the activity of desaturases, particularly those acting on the essential polyunsaturated fatty acids (i.e., the Δ6-, Δ5-, and Δ4-desaturases), possibly involves regulation at the level of enzyme biosynthesis (Wahle, 1983; de Gomez Dumm et al., 1983). Rat liver microsomal membrane desaturase activity also appears to be regulated by dietary lipids independently of other membrane-associated lipid-metabolizing enzymes, such as fatty acyl-CoA:*sn*-glycero-3-phosphocholine acyl transferase (ACAT; Pugh and Kates, 1984). Both membrane lipid composition and the activity of various membrane-associated lipid-metabolizing enzymes change rapidly in response to dietary lipid supplementation. Thus, within 12 h of dietary supplementation with safflower oil and cholesterol, HMG-CoA reductase and ACAT activities were significantly altered (Mitropoulos et al., 1980).

Dietary lipid effects on mitochondrial respiration have attracted considerable attention because of the central role of electron transport and oxidative phosphorylation in the cell and the membrane-associated nature of these processes. Early studies established that the membrane lipid composition of mammalian mitochondria was influenced by a dietary deficiency of essential fatty acids. A number of key mitochondrial respiratory enzymes and membrane functions were suppressed following the administration of essential fatty acid-deficient diets and were overcome by resupplying the fatty acids 18:2 n-6 and 18:3 n-3 in the diet (Haeffner and Privett, 1975; Williams et al., 1975). Recent studies have clearly established that mitochondrial membrane lipid composition and enzyme function are also influenced by dietary lipids, even when the diet is sufficient in essential fatty acids. Some of these effects have been reported only for mitochondria isolated from liver (Rafael et al., 1984), but, in general, mitochondria from skeletal and cardiac muscle also appear susceptible to dietary lipid manipulation.

Oxidation of succinate, glutamate, α-ketoglutarate, and palmitoyl carnitine (plus malate) by the mitochondrial respiratory chain is influenced by diet-induced changes in membrane lipid composition. Measurement of both state 3 and state 4 respiratory rates for oxidation of these substrates has been popular for determining the effect of dietary lipids on mitochondrial "intactness" (by determining the respiratory control ratio), the coupling efficiency (ADP/O ratio), and the rate of ATP synthesis inferred from measurement of the state 3 respiratory rate (ADP, nonlimiting; Clandinin, 1978; Mak et al., 1983; Blomstrand and Svensson, 1983; McMurchie et al., 1984a). The effects of dietary lipid manipulation appear to be dependent on the site of substrate entry into the electron transport chain, indicating that various mitochondrial electron transport reactions may exhibit differential sensitivity to the effects of changes in membrane lipid composition mediated by dietary lipids. The apparent dependence of some of these reaction sequences on particular membrane lipids (Daum, 1985) may partially explain the diverse effects reported. For example, dietary 22:1 cis fatty acids, such as cetoleic and erucic acids originating from fish oil and rapeseed oil, respectively, specifically interfere with the fatty acid composition of cardiolipin in rat and chicken heart mitochondria (Renner et al., 1979; Blomstrand and Svensson, 1983). They also result in the inhibition of mitochondrial oxidative phosphorylation and ATP synthesis. Whereas analysis of the fatty acid composition of individual phospholipids provides information about the extent to which dietary lipids affect the different classes of membrane phospholipids, it would not be possible from such analysis to determine accurately the effect of compositional changes on individual enzyme reactions involved in mitochondrial oxidative phosphorylation (Innis and Clandinin, 1981c). Some studies investigating the role of dietary lipids on mitochondrial respiratory properties have reported minimal effects (Royce and Holmes, 1984). However, in light of other studies contrasting with such an observation, it appears that the ability of mitochondrial membranes to adapt and to compensate for possible deleterious effects of dietary lipids on functional properties provides a likely explanation for these contrasting observations.

Dietary lipids have been reported to affect mitochondrial $F_1$-ATPase by influencing both specific activity (as opposed to state 3 rates of respiration) and sensitivity to activators and inhibitors. McMurchie et al. (1983a) reported that the oligomycin-sensitive $F_1$-ATPase activity of rat heart mitochondria was increased with dietary sheep kidney fat and was decreased with dietary sunflower seed oil, relative to a low-fat, reference diet. This supports the results of Robblee and Clandinin (1984), showing that mitochondrial ATPase activity increased upon lowering the P/S ratio of the diet. Decreasing the level of fat, irrespective of its P/S ratio, also increased mitochondrial $F_1$-ATPase activity (Robblee and Clandinin, 1984). One of the reactions of

mitochondrial ATPase, the ATP/$^{32}$P exchange reaction, was also reported to be sensitive to dietary fat, with safflower oil decreasing the activity of this reaction in rat liver mitochondria in comparison to beef tallow (Abuirmeileh and Elson, 1980b). In addition to diet-induced changes in the specific activity of $F_1$-ATPase, the interactions of the $F_1$-ATPase inhibitor oligomycin and the $F_1$-ATPase stimulator 2,4-DNP were influenced by the nature of the dietary fat (Zsigmond and Clandinin, 1986). This study (Zsigmond and Clandinin, 1986) indicated that dietary lipids affected the catalytic activity of the mitochondrial ATPases to both uncouplers and inhibitors by altering the composition of the hydrophobic fatty acyl chains and/or the polar head groups in the region of the ATPase enzyme complex.

A number of membrane-associated hormone receptor systems that regulate key metabolic processes appear to be influenced by changes in membrane lipid composition induced by dietary lipids. For example, dietary linoleic acid modulates rat liver plasma membrane unsaturated fatty acid composition, phosphatidylcholine and cholesterol contents and glucagon-stimulated adenylyl cyclase activity (Neelands and Clandinin, 1983; Morson and Clandinin, 1985). The activities of 5′-nucleotidase and ouabain-sensitive ($Na^+$, $K^+$)-ATPase of rat liver plasma membranes also appear to be modulated by dietary lipids (Brivio-Haugland et al., 1976), as does the specific binding of insulin to rat liver plasma membranes (Sun et al., 1977). The recent observation that dietary lipids induce changes in the lipid composition of mouse liver nuclear envelopes, the activity of nucleoside triphosphatase, and the binding of L-triiodothyronine associated with this membrane fraction (Venkatraman et al., 1986) raises the interesting possibility that dietary lipids influence enzyme biosynthesis by their effects on both binding and transport of mRNA to the nuclear envelope.

Dietary saturated fat can also exert inhibitory effects on hormone-stimulated lipolysis by influencing several points in the lipolytic cascade. Thus the activity of both norepinephrine-stimulated adenylyl cyclase and cAMP phosphodiesterase is influenced by dietary lipids (Awad and Chattopadhyay, 1986b), although hormone-sensitive lipase activity is not affected (Awad and Chattopadhyay, 1986a). Some of these effects may be mediated by diet-induced changes in the affinity ($k_d$) and number ($B_{max}$) of β-adrenergic receptors located on the plasma membrane of adipocytes (Awad, 1983). Other dietary lipid effects on adipocytes could be mediated by changes in insulin binding parameters (van Amelsvoort et al., 1986).

The nutritional link between dietary lipids and coronary heart disease has traditionally emphasized the role of lipids in both atherogenic and thrombogenic processes. Much less attention has been paid to the possibility that dietary lipids influence cardiac function in a more direct manner by affecting the properties of the membrane systems instrumental in controlling the contractile cycle of the heart. Dietary lipids significantly influence cardiac mitochondrial membranes. Changes have been reported in membrane lipid composition (Table 7–1), membrane biophysical properties (Table 7–2), and mitochondrial respiration (Table 7–4). Although it is probable that dietary lipid manipulation could affect the ability of cardiac mitochondria to produce ATP under certain conditions, the effects of dietary lipids on cardiac membranes other than those of the mitochondrion are more likely to induce changes in cardiac contractility. The cell surface or sarcolemmal membrane of the mammalian heart is the site of many receptors, enzymes, and ion channels, and in an integrated fashion these membrane processes play a dominant role in controlling the beat-to-beat regulation of the heart as dictated by a variety of hormones and stimuli. The β-adrenergic adenylyl cyclase system, the calcium ion channel, and the ($Na^+$,$K^+$)-ATPase associated with the sarcolemmal membrane are in part responsible for the control of cardiac contractility. The sarcoplasmic reticulum also plays a prominent role in the contractile cycle by its ability to control

the intracellular level of calcium ions. Therefore, these membrane systems and their associated functional processes are likely targets for modulation by dietary lipids. If these membrane systems are subject to the influences of dietary lipids, cardiac function is likely to be compromised because of the central role these processes play in the control of cardiac contractility. For example, the nature of the dietary lipid intake may influence the susceptibility of the heart to the development of arrhythmias under the influence of calcium ions and catecholamines (Charnock et al., 1985b).

Changes in the membrane phospholipid fatty acid composition of cardiac sarcolemmal-enriched membrane fractions are similar to those reported for heart mitochondria of the rat and the marmoset monkey following dietary sunflower seed oil or sheep kidney fat supplementation (McMurchie, 1986; McMurchie et al., 1987, 1988). However, in the sarcolemmal membrane-enriched fraction, significant changes were apparent in the membrane cholesterol to phospholipid (C/PL) ratio, which in the rat was elevated only by dietary cholesterol. This is in contrast to the marmoset monkey, in which feeding a sheep kidney fat diet without cholesterol increased the membrane C/PL ratio. For both the rat and the marmoset monkey, the increased membrane C/PL ratio was accompanied by an increased responsiveness (but not sensitivity) of the β-adrenergic receptor adenylyl cyclase system to stimulation by catecholamines (McMurchie, 1986; McMurchie et al., 1987, 1988). This observation is significant in terms of the effect of dietary lipids on the inotropic and chronotropic responses of the heart to catecholamines mediated by the β-adrenergic adenylyl cyclase system and the possible induction of tachyarrhythmias. Equally important is the protective role of dietary sunflower seed oil in preventing tachyarrhythmias following catecholamine stimulation. The lack of an effect of dietary fatty acid supplementation on the β-adrenergic adenylyl cyclase system of the rat heart reported by McMurchie (1986) and McMurchie et al. (1987) contrasts with the results of Wince and Rutledge (1981). They reported that dietary coconut oil increased atrial catecholamine-stimulated adenylyl cyclase activity, whereas dietary sunflower seed oil influenced the binding parameters of a β-adrenergic specific radioligand. A number of factors, including the use of atrial as opposed to ventricular tissue and dietary coconut oil in contrast to sheep kidney fat, may account for differences between the two studies. Furthermore, in the study by Wince and Rutledge (1981), the membrane C/PL ratio was not determined, and it is possible that changes in this ratio accounted for their reported effects. Overall, the results reinforce the previously mentioned observation that hormone-sensitive adenylyl cyclases are sensitive to their membrane lipid environment and that changes in the membrane C/PL ratio are one means by which the activity of this enzyme–receptor complex may be modified by dietary lipids.

Dietary lipid changes at the level of the sarcolemmal membrane could be manifest at the physiological/pharmacological level in terms of an alteration in the contractile ability of the heart. Indeed, the effect of dietary sunflower seed oil on increasing coronary flow and ventricular work (Ten Hoor et al., 1973; de Deckere and Ten Hoor, 1980) may be the result of dietary-induced changes in the control of sarcolemmal membrane-associated processes mediated by changes in membrane physical properties. The role of membrane unsaturated fatty acids, particularly those of the n-3 series in regulating the response of the heart to catecholamines (Gudbjarnason et al., 1978), may involve changes in sarcolemmal membrane-associated adrenergic receptor function. However, it is possible that dietary lipids induce changes in mammalian cardiac function by changing the balance and/or production of prostaglandins and thromboxanes (Hoffman, 1986).

Erythrocyte membranes are a popular system to use when examining the effects of dietary lipids on membrane lipid properties and the subsequent modulation of membrane-

associated enzyme activity. The allosteric behavior of erythrocyte membrane-bound ATPases and acetylcholinesterase to various activators and inhibitors following dietary lipid supplementation has been measured by determining the Hill coefficient. Changes in the value of the Hill coefficient are considered indicative of changes in the extent of cooperativity. Dietary lipid modification of the lipid composition of rat erythrocyte membranes has been correlated with changes in the allosteric behavior of $(Na^+,K^+)$-ATPase and acetylcholinesterase to inhibition by fluoride ions (Bloj et al., 1973b). However, for these enzymes, similar shifts in membrane lipid fluidity (inferred from the value of the double bond index to saturated fatty acid ratio) resulted in opposite shifts in the value of the Hill coefficient. The implication of this finding was that membrane enzymes located within the same membrane could behave differently in terms of allosteric modification as a result of the effect of dietary lipids (Bloj et al., 1973b). The intrinsically high C/PL ratio of the rat erythrocyte membrane may be responsible for opposing the effects dietary lipids exert on the allosteric behavior of various membrane-associated enzymes (Bloj et al., 1973a,b, 1976). For example, the kinetic behavior of acetylcholinesterase to fluoride ($F^-$) inhibition and $(Ca^{2+},Mg^{2+})$-ATPase to $Ca^{2+}$ activation was modified by dietary cholesterol only when these enzymes were associated with membranes having a high (inferred) lipid fluidity. Modification, therefore, occurred following dietary corn oil supplementation, but not when the membrane fluidity was relatively low, as was inferred following dietary supplementation with lard or hydrogenated fat (Bloj et al., 1976). Increasing the fluidity of rat erythrocyte membranes by dietary corn oil supplementation also allowed insulin and epinephrine to modify the allosteric behavior of membrane-bound acetylcholinesterase to inhibition by $F^-$, a result not obtained when dietary saturated fatty acids were supplied (de Melián et al., 1978). These results emphasize that, for dietary lipids to produce significant effects on functional activity via changes in membrane lipid fluidity, the initial fluidity of the particular membrane is important.

Other parameters associated with erythrocytes, as well as the properties of platelets, are influenced by dietary lipids. For example, the fragility of erythrocytes is altered by dietary lipids particularly following an increase in membrane 18:1 cis content (Benga et al., 1984). The rheological properties of human blood correlate with the total unsaturation of erythrocyte membrane phospholipids following dietary lipid supplementation, indicating a role for erythrocyte membrane fluidity in determining blood viscosity (Cartwright et al., 1985). Although platelet reactivity can be influenced by the balance of various prostacyclins and thromboxanes, there is evidence that dietary lipids also influence platelet function by their effects on platelet (plasma) membrane fluidity in various species, including man (McGregor et al., 1981; Vas Dias et al., 1982; Terano et al., 1983).

The results reported under Miscellaneous Effects in Table 7–4 are final testimony to the diversity of effects that dietary lipid changes can produce. For example, the uptake of lipids from the small intestine is selectively altered by the lipid composition of the intestinal brush border membrane (Thompson et al., 1986), indicative of a possible feedback control by dietary lipids on fat adsorption at the level of the intestinal membrane barrier. Finally, the effects of ethanol (John et al., 1980) and volatile anesthetics (Evers et al., 1986) on various behavioral responses are modulated by changes in cell membrane lipid composition mediated by the nature of the dietary lipid intake. Although these results have so far been reported only for rodents, they may be equally valid for man.

## CONCLUSIONS AND PERSPECTIVES

There is now no reason to doubt that the philosophy reflected in the saying "We are what we eat" can be directly and unequivocally applied at the level of cellular mem-

brane structure and function. In this instance, the nutritional link is by way of dietary lipids and their influence on membrane lipid composition; the structural link is the intimate physical association between lipids and proteins in biological membranes, whereas the functional link is provided by the host of metabolic processes catalyzed by membrane-associated proteins. Perturbation of many of the functional activities associated with membranes has been recognized as a major contributor to various aspects of cell dysfunction. In the context of the "diseases of affluence" and their nutritional link with lipids, which is becoming increasingly evident in developed western societies, the role of the membrane and the influence of dietary lipids on the properties of this cell structure must now be considered a primary target for preventive and even therapeutic interventions.

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