Dietary fats and membrane function: implications for metabolism and disease

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Abstract

Lipids play varied and critical roles in metabolism, with function dramatically modulated by the individual fatty acid moities in complex lipid entities. In particular, the fatty acid composition of membrane lipids greatly influences membrane function. Here we consider the role of dietary fatty acid profile on membrane composition and, in turn, its impact on prevalent disease clusters of the metabolic syndrome and mental illness. Applying the classical physiological conformer-regulator paradigm to quantify the influence of dietary fats on membrane lipid composition (i.e. where the membrane variable is plotted against the same variable in the environment - in this case dietary fats), membrane lipid composition appears as a predominantly regulated parameter. Membranes remain relatively constant in their saturated (SFA) and monounsaturated (MUFA) fatty acid levels over a wide range of dietary variation for these fatty acids. Membrane composition was found to be more responsive to n-6 and n-3 polyunsaturated fatty acid (PUFA) levels in the diet and most sensitive to n-3 PUFA and to the n-3/n-6 ratio. These differential responses are probably due to the fact that both n-6 and n-3 PUFA classes cannot be synthesised *de novo* by higher animals. Diet-induced modifications in membrane lipid composition are associated with changes in the rates of membrane-linked cellular processes that are major contributors to energy metabolism. For example, in the intrinsic activity of fundamental processes such as the Na^+/K^+ pump and proton pump-leak cycle. Equally, dietary lipid profile impacts substantially on diseases of the metabolic syndrome with evidence accruing for changes in metabolic rate and neuropeptide regulation (thus influencing both sides of the energy balance equation), in second messenger generation and in gene expression influencing a range of glucose and lipid handling pathways. Finally, there is a growing literature relating changes in dietary fatty acid profile to many aspects of mental health. The understanding of dietary lipid profile and its influence on membrane function in relation to metabolic dysregulation has exciting potential for the prevention and treatment of a range of prevalent disease states.

Keywords

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Abstract

Objective: *Lipids play varied and critical roles in metabolism, with function dramatically modulated by the individual fatty acid moities in complex lipid entities. In particular, the fatty acid composition of membrane lipids greatly influences membrane function. Here we consider the role of dietary fatty acid profile on membrane composition and, in turn, its impact on prevalent disease clusters of the metabolic syndrome and mental illness. Applying the classical physiological conformer-regulator paradigm to quantify the influence of dietary fats on membrane lipid composition (i.e. where the membrane variable is plotted against the same variable in the environment - in this case dietary fats), membrane lipid composition appears as a predominantly regulated parameter. Membranes remain relatively constant in their saturated (SFA) and monounsaturated (MUFA) fatty acid levels over a wide range of dietary variation for these fatty acids. Membrane composition was found to be more responsive to n-6 and n-3 polyunsaturated fatty acid (PUFA) levels in the diet and most sensitive to n-3 PUFA and to the n-3/n-6 ratio. These differential responses are probably due to the fact that both n-6 and n-3 PUFA classes cannot be synthesised de novo by higher animals. Diet-induced modifications in membrane lipid composition are associated with changes in the rates of membrane-linked cellular processes that are major contributors to energy metabolism. For example, in the intrinsic activity of fundamental processes such as the Na^+/K^+ pump and proton pump-leak cycle. Equally, dietary lipid profile impacts substantially on diseases of the metabolic syndrome with evidence accruing for changes in metabolic rate and neuropeptide regulation (thus influencing both sides of the energy balance equation), in second messenger generation and in gene expression influencing a range of glucose and lipid handling pathways. Finally, there is a growing literature relating changes in dietary fatty acid profile to many aspects of mental health. The understanding of dietary lipid profile and its influence on membrane function in relation to metabolic dysregulation has exciting potential for the prevention and treatment of a range of prevalent disease states.*

1. INTRODUCTION

'You are what you eat', like many aphorisms is both true and untrue. Animals are descended from the branch of prokaryotes that consumed other organisms or their organic products. We are heterotrophs and as well as requiring organic molecules as our source of energy, we also require some specific molecules preformed. Presumably, this is due to the loss of functional enzymatic systems for their synthesis at various times in our ancestry. The evolutionary loss of this synthetic ability meant that these molecules then became essential requirements for the species that followed. For most animals, these essential components of diet can be grouped into vitamins, amino acids and fatty acids. Whilst some mammals have symbiotic relations with gut micro-organisms to synthesise the essential nutrients and then provide them to their host, for most mammals, including humans, they need to be obtained preformed in the diet. In the case of both amino acids and fatty acids, only some are required preformed and other members of the class can be synthesised from these once assimilated. The first demonstration of the essential requirement for fat in the diet was in 1929, when Burr & Burr (Burr & Burr, 1929) demonstrated that mice reared on a fat-free diet failed to grow, developed pathologies and subsequently died.

In this contribution, we will be concerned only with fats and specifically with the role that dietary fat composition has on the function of the animals that ingest them. The evidence we will discuss is largely derived from mammals, especially rats and humans. However, evidence comes from a range of animals and has broad implications across species. The importance of dietary fats is primarily due to the fact that they provide the backbone of membranes, an essential component of all life. If DNA can be described as an 'eternal' molecule of life, then membranes can be described as an 'eternal' structure of life, as existing membranes are formed from pre-existing membranes. Apart from providing the semipermeable barriers between cells and the external environment, membranes also partition animal cells into essential sub-cellular compartments. The lipid membrane bilayer also provides a dynamic environment in which much of the important metabolic chemistry of life occurs. With substantial molecular variety, membrane lipid composition is both adaptive and highly varied. Here we will concentrate on the types of dietary fats, their influence on membrane composition and consequently membrane function, with the flow-on effects on metabolism and two diseases of increasing incidence in the modern world: the diseases associated with the 'metabolic syndrome' and 'mental illness'.

2. TYPES AND PROPERTIES OF FATTY ACIDS

As universal features of eukaryotic life, lipid bilayers are assemblies of amphipathic lipid molecules held together by non-covalent bonds with the outer surfaces of membrane bilayers consisting of charged headgroups and the inner hydrocarbon core consisting primarily of long hydrocarbon acyl chains. The main types of fatty acyl chains found both in the diet of mammals and as part of mammalian membrane lipids are listed in Table 1. These fatty acyl chains, or fatty acids, can be categorised as either saturates (SFAs), monounsaturates (MUFAs), or polyunsaturates (PUFAs), with the PUFAs being further divided into omega-3 (n-3), omega-6 (n-6) and some omega-9 (n-9). As illustrated in Table 1 and Fig. 1, fatty acids can be individually identified by a numerical representation using the number of carbon atoms: number of double bonds and position of the first double bond relative to the methyl end of the molecule. The type of double bonds in naturally occurring fatty acyl chains are of the *cis*-configuration. Fig. 1 illustrates the chemical structure of a representative triglyceride and phospholipid.
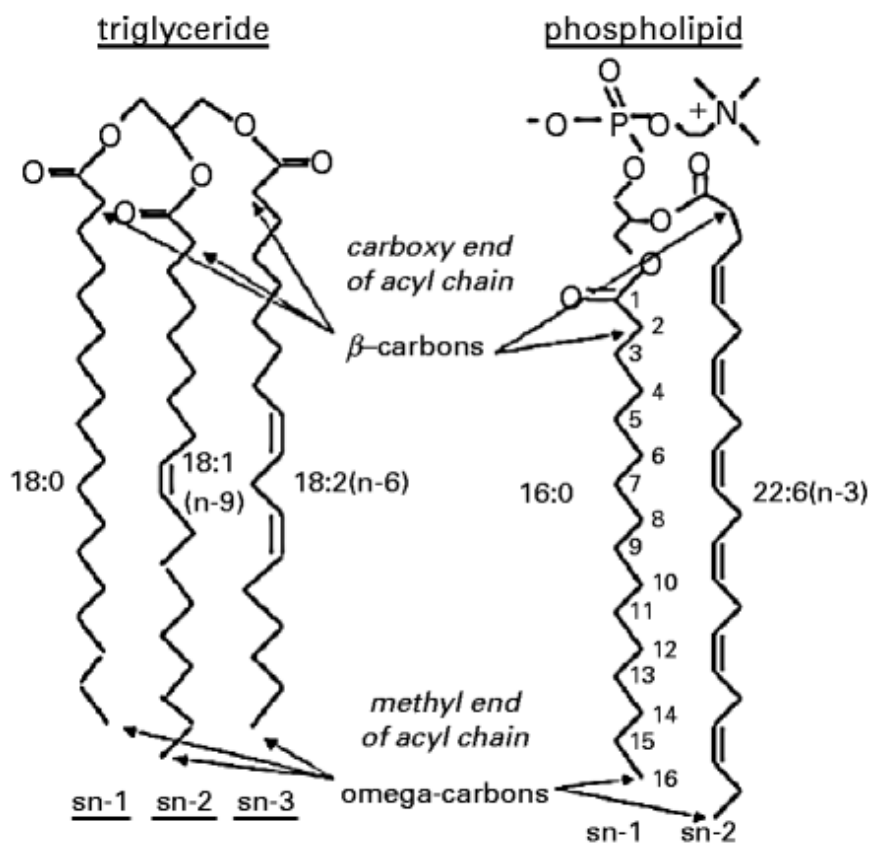
Fig. 1. Schematic diagram illustrating the chemical structure of a representative triglyceride and a representative phospholipid. The triglyceride shown is 1-stearoyl, 2 oleoyl, 3-linoleoyl glycerol. The phospholipid is 1-palmitoyl, 2-docosahexaenoyl phosphatidylcholine. The sn-1, sn-2, and sn-3 acyl chains are labelled for each lipid molecule. The beta-(β-) carbons at the carboxy end and the omega (n-) carbons at the methyl end of each fatty acyl chain are identified. For the palmitoyl acyl chain in the phospholipid the individual carbons in this 16:0 acyl chains are also numbered.

Both the chain length and the number of double bonds in these acyl chains have a major influence on the physical properties of the lipids that contain them. For example, phosphatidylcholine (PC) molecules with 18:0 acyl chains in both the sn-1 and sn-2 positions (sn refers to stereospecific numbering of carbons with the polar head group being attached to carbon number 3 as shown in Fig. 1) have a melting point of 55°C and thus would be solid at mammalian body temperatures. If 18:1 n-9 is substituted for 18:0 in the sn-2 position, the melting point decreases to approximately 1°C and thus would be liquid-crystalline at mammalian body temperatures. If the sn-2 acyl chain was further changed to 18:2 n-6, the liquid crystalline state would be maintained to approximately −15°C (Lee, 1991). However, substitution of acyl chains with greater degrees of polyunsaturation in the sn-2 position does not further lower but rather results in a small increase of the phase transition temperature. It does however result in a further increase in the energy changes associated with the phase transition (Niebylski & Salem Jr, 1994). This dramatic change in melting point with only small changes in molecular structure is indicative of the large changes in the molecular dynamics of these acyl chains. As the acyl chain length of membrane lipids averages about 18 carbons, the presumed evolutionary reason why membrane lipids do not have saturated acyl chains in both the sn-1 and sn-2 positions is due to these physical properties. Whilst the sn-2

position of a membrane lipid is always an unsaturated acyl chain, the sn-1 position can be either a saturated or an unsaturated acyl chain. Therefore the nature of the fatty acyl chains that constitute membrane lipids have substantial and significant effects on the fluidity and other dynamic properties of membranes (for a review see Cribier, Morot & Zachowski, 1993; and for some emerging areas see Pike, 2003; Stillwell & Wassall, 2003) and thus a substantial and significant influence on membrane function

Table1: Some fatty acyl chains found in membrane bilayers of animal cells

Common name	Systematic name	Abbreviation
Saturates		
myristic	tetradecanoic	14:0
palmitic	hexadecanoic	16:0
stearic	octadecanoic	18:0
arachidic	eicosadecanoic	20:0
Unsaturates		
Monounsaturates		
palmitoleic	*cis*-9-hexadecenoic*	16:1 n-7**
vaccenic	*cis*-11-octadecenoic	18:1 n-7
oleic	*cis*-9-octadecenoic	18:1 n-9
Polyunsaturates		
omega-9		
mead	all-*cis*-5,8,11-eicosatrienoic	20:3 n-9
omega-6		
α-linoleic	*cis,cis*-9,12-octadecadienoic	18:2 n-6
γ-linolenic	all-*cis*-6,9,12-octadecatrienoic	18:3 n-6
No common name	all-*cis*-8,11,14-eicosatrienoic	20:3 n-6
arachidonic	all-*cis*-5,8,11,14-eicosatetraenoic	20:4 n-6
No common name	all-*cis*-4,7,10,13,16-docosapentaenoic	22:5 n-6
omega-3		
α-linolenic	all-*cis*-9,12,15-octadecatrienoic	18:3 n-3
No common name	all-*cis*-11,14,17-eicosatrienoic	20:3 n-3
EPA	all-*cis*-5,8,11,14,17-eicosapentaenoic	20:5 n-3
DHA	all-*cis*-4,7,10,13,16,19-docosahexaenoic	22:6 n-3

* number indicate the position of double bonds numbered from carboxylic end.
** the number preceding the colon indicates the number of carbon atoms; the number following the colon indicates the number of double bonds; the position of the double bond nearest the methyl end is given by the designation n (number of carbon atoms in from the methyl end of the chain).
EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

3. SOURCE AND FATE OF DIETARY FATTY ACIDS

Whilst animals can synthesise both saturated (SFA) and monounsaturated (MUFA) acyl chains from acetyl coenzyme A (acetyl CoA) (and thus from non-lipid sources), higher animals are unable to synthesise *de novo* either n-6 or n-3 polyunsaturated fatty acids (PUFAs). The fatty acids in these two groups must be obtained either from the diet or synthesised by gut micro-organisms, with n-6 and n-3 PUFAs, such as 18:2 n-6 and 18:3 n-3, able to be elongated and further desaturated to make longer chain n-6 and n-3 PUFAs. These desaturase and elongase enzyme systems in animal tissues can also elongate and desaturate the monounsaturated oleic acid (18:1 n-9) to produce the polyunsaturated mead acid (20:3 n-9) but they have a substrate preference for 18 carbon n-3 and n-6 PUFA over 18:1 n-9. Thus 20:3 n-9 is normally not produced in any quantity and its presence in significant amounts in membranes is normally indicative of n-3 and n-6 PUFA dietary deficiency. If n-3 PUFAs are deficient but n-6 PUFAs are sufficient in the diet there is often a significant amount of n-6 PUFAs longer and more desaturated than 20:4 n-6 in membrane lipids.

Both the fat content and the fatty acid composition of foodstuffs vary widely. Saturated and monounsaturated fatty acids are found in food from both animal and plant sources.

Polyunsaturates from plant sources consist primarily of 18 carbon acyl chains rather than the 20–22 carbon acyl chains. While vegetable oils derived from plant seeds are high in linoleic acid (LA; n-6 PUFA), green leaf food sources are generally high in α-linolenic acid (LNA; n-3 PUFA) because this fatty acid is common in chloroplast membranes. For example, the ratio of 18:3 n-3 / 18:2 n-6 in green vegetables varies from 4.6 in spinach to 0.2 in peas; with broccoli, green beans, cabbage, romaine lettuce and iceberg lettuce having intermediate values of 2.8, 1.7, 1.6, 2.7 and 0.6, respectively (Beare-Rogers, 1989). Breads generally have ratios less than 0.1. Vegetable oils being rich in 18:2 n-6 also have low ratios, for example safflower oil has essentially no n-3 PUFA with a n-3/n-6 ratio of less than 0.005. Canola oil, regarded as a high n-3 PUFA oil has a ratio of 0.5 whilst probably the most common vegetable oil in the modern human diet, soybean oil, has a value of 0.1 (Beare-Rogers, 1989). Once assimilated by animals, these two PUFAs (18:3 n-3 and 18:2 n-6) can be converted to the more unsaturated long chain PUFAs (e.g. 22:6 n-3 and 20:4 n-6) by the elongase and desaturase enzyme systems. The synthesis of the long chain 22:6 n-3 differs from and is more complicated than that of 20:4 n-6 in that it also involves a single cycle of β-oxidation in peroxisomes (see Sprecher, Chen & Yin, 1999). Preformed long chain n-6 and n-3 PUFAs in the diet will be primarily obtained from animal tissues. Fish (especially cold water fish) and brain are food sources high in long chain n-3 PUFAs whilst muscle and kidney, as food, are high in long chain n-6 PUFAs. The meat of wild ruminants contains much higher levels of PUFAs than meat from domestic cattle. This is especially true for n-3 PUFAs with the ratio of n-3/n-6 PUFA approximately 0.4 for meat from wild ruminants and pasture-fed cattle but significantly less for grain-fed cattle at <0.2 (Cordain *et al.*, 2002). This difference might seem small but together with the dominance of soybean oil in the manufactured foods eaten by many modern humans, may have significant health implications for people in developed countries (see later discussion).

Once ingested and absorbed, fatty acids are either incorporated into membrane lipids, into storage lipids (triglycerides) or directly oxidised to CO_2, H_2O and energy. The feeding of different fatty acids, labelled with carbon isotopes, to both rats and humans has revealed differences in their tendency to be oxidised. In a recent study on humans (Delany *et al.*, 2000), the exhalation of labelled-$^{14}CO_2$ peaked at 4–6 h after ingestion of the fatty acids and declined such that by 9 h most of the release of labelled $^{14}CO_2$ was complete. A greater amount of labelled $^{14}CO_2$ was exhaled when the carboxy-end of the fatty acid contained the isotope than when the omega methyl carbon was labelled, which is understandable since oxidation of fats occurs sequentially at the β-carbon, that is, at the carboxy-end of the molecule. In humans the cumulative amount of labelled $^{14}CO_2$ exhaled over 9 h post-ingestion averaged 11% for the saturated 18:0. The amount of ingested fatty acid that underwent oxidation was inversely related to chain length but directly related to degree of unsaturation (i.e. relative number of carbon double bonds). It was greatest for the short-chain saturate (approximately 34% for 12:0) and increased for unsaturated fats of the same chain length (17% for 18:1 n-9; 16% for 18:2 n-6 and 24% for 18:3 n-3). In the case of oleic acid (18:1 n-9) there was no significant difference between the *cis*- and *trans*-isomers (Delany *et al.*, 2000). These relative differences are similar to results previously obtained in rats (Leyton, Drury & Crawford, 1987) and indicate that the majority of ingested fatty acids are probably incorporated into either storage triglycerides or membrane lipids.


4. MEMBRANE RESPONSIVENESS TO DIETARY FAT COMPOSITION: 'REGULATORS' OR 'CONFORMERS'?

Although there are hundreds of different molecular phospholipid species in the cellular membranes of rat hepatocytes, only four molecular species of phosphatidylcholine and phosphatidylethanolamine (PE; 16:0/18:1, 16:0/18:2, 16:0/22:6 and 18:1/18:2) are synthesised *de novo*. All other molecular species are the result of the remodelling of these *de novo*-produced phospholipid molecules by various deacylation/reacylation processes (Schmid, Deli & Schmid, 1995). The relative constancy of membrane lipid composition, as with many cellular components, hides an environment with rapid turnover and rapid remodelling. This is presumably an evolutionarily old property of membranes as bacteria (*Escherichia coli*) transferred to a low temperature replace 16:0 acyl chains in their phospholipids with 18:1 n-7 acyl chains, producing membrane lipids with two monounsaturated acyl chains. This response is measurable within 30s of transfer and results in constant membrane fluidity, a process termed 'homeoviscous' adaptation (Rock, Jackowski & Cronan, 1996). Eukaryotic cells also show rapid turnover of membrane lipids. For example, labelled (^{14}C) fatty acids added to culture media are processed through the endoplasmic reticulum and appear in the plasma membranes within 2–10min after addition (Chakravarthy, Spence & Cook, 1986).

Despite the rapid turnover of membrane lipids, changes in the fatty acid composition of the diet can have varying effects on the fatty acid composition of membrane lipids. Fig. 2 shows an analysis of the influence of dietary fatty acid composition on the fatty acid composition of four specific cellular membranes in the rat. The membrane systems presented are the hepatocyte plasmalemma, cardiac sarcolemma, cerebral synaptosomes and cerebral myelin. The analysis uses the classic physiological 'conformer/regulator' paradigm to quantify the influence of dietary fatty acid composition on membrane fatty acid composition. In this paradigm, the membrane variable is plotted against the same variable for the environment (in this case the diet) and the slope of the relationship determined. A 'perfect membrane conformer' will have a slope of 1 as the membrane parameter will be directly related to variation in the same environmental (i.e. diet) parameter. A 'perfect membrane regulator' will have a slope of 0 as the membrane parameter remains constant irrespective of changes in the same parameter in the diet.

The analysis shows that although there were very large differences in the relative content of saturated fatty acids in the different diets, the percent saturation of membrane lipids was relatively constant at around 50% in all four cellular membranes (i.e slopes of −0.05 to 0.01, see Fig. 2). Similarly, although there was considerable variation in the degree of monounsaturation of the dietary fats, the degree of monounsaturation of the membrane lipids remained relatively constant (i.e. slopes of −0.07 to 0.11). The n-6 PUFA content of the membranes showed a greater response to variation in dietary n-6 PUFA content than observed for the monounsaturates, although this could still be characterised as a relatively regulated membrane variable. The response of the different membranes varied from a 7% increase in cerebral synaptosome membrane n-6 PUFA for a 100% increase in dietary n-6 PUFA, to a 22% increase in hepatocyte plasmalemmal n-6 PUFA content.
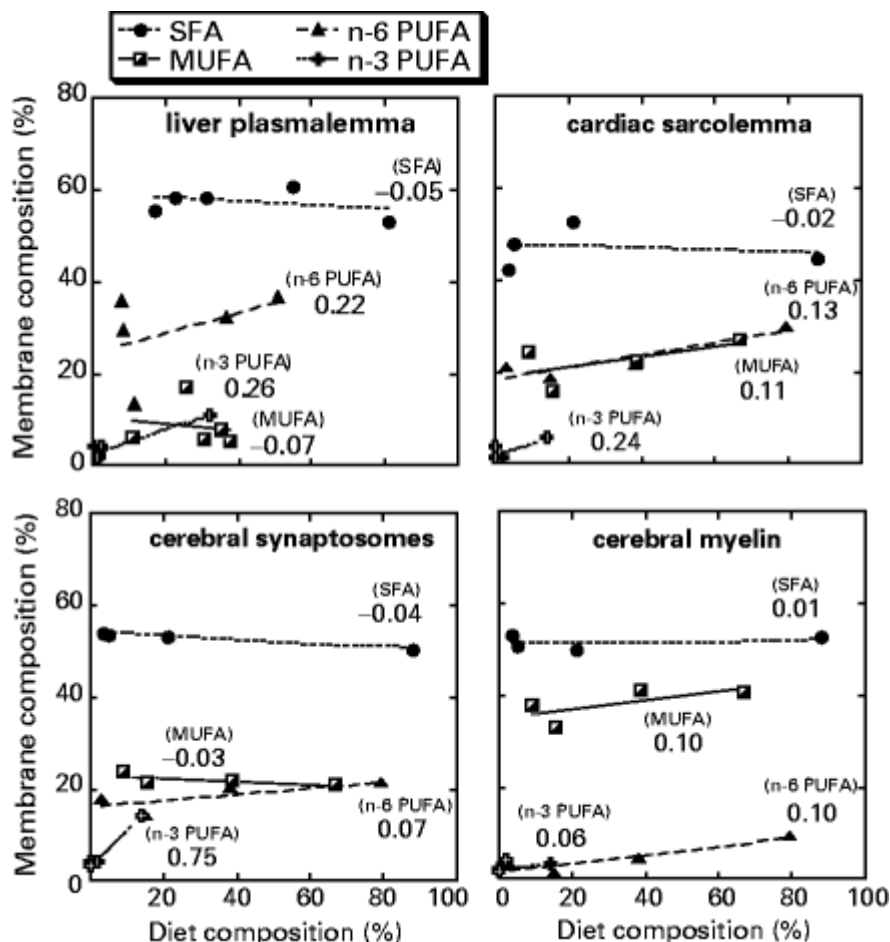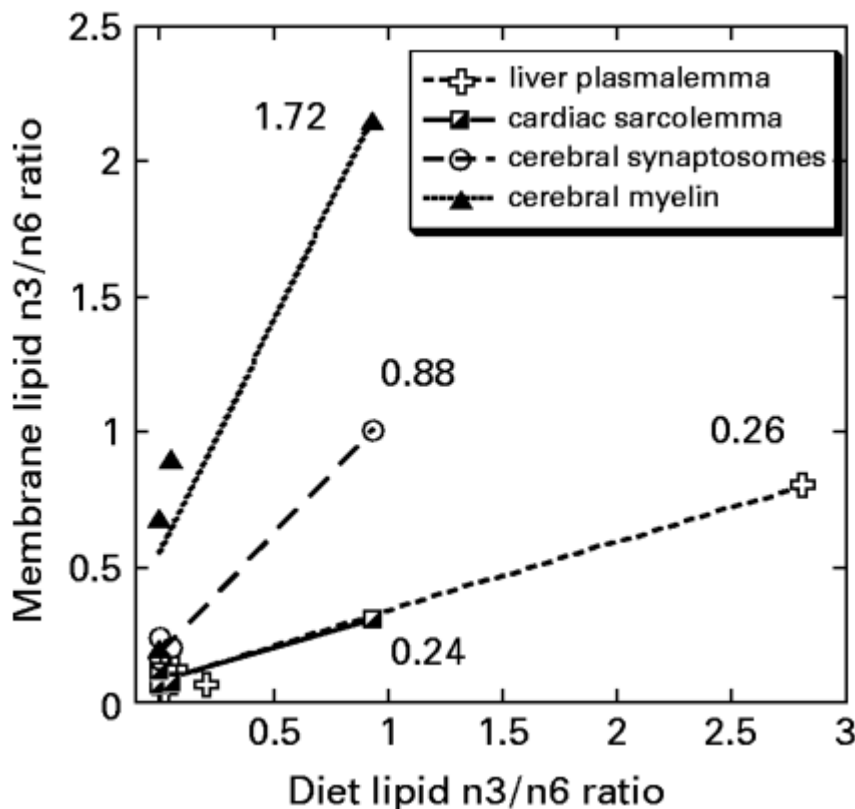
Fig. 2: The relationship between diet fatty acid composition and the fatty acid composition of four different rat membrane preparations (liver plasmalemma, cardiac sarcolemma, cerebral synaptosomes and cerebral myelin). The number to the right of each line is the slope of the relationship and represents the degree of responsiveness of membrane lipid composition to diet composition. A value of 0 signifies no influence of dietary fatty acid composition on phospholipid fatty acid composition whilst a value of 1.0 signifies that for the particular fatty acid parameter there is a 100% concordance between change in diet and tissue phospholipid composition. Data for these analysis for liver plasmalemma from Clamp *et al.* (1997), for cardiac sarcolemma are from Vajreswari & Narayanareddy (1992) and for cerebral synaptosomes and myelin are from Srinivasarao *et al*. (1997). SFA is saturated, MUFA is monounsaturated and n-6 and n-3 PUFA are polyunsaturated fatty acids.

The greatest sensitivity to changes in dietary fatty acids was for n-3 PUFA. Membrane fatty acid composition varied from a 6% change in cerebral myelin up to a 75% change in cerebral synaptosomes in response to a 100% change in dietary n-3 PUFA. Despite coming from different studies, the response of liver and heart plasma membrane preparations to variation in dietary n-3 PUFA content were very similar. For a 100% change in dietary n-3 PUFA, there was a 24% and 26% change in n-3 PUFA content of cardiac sarcolemma and liver plasmalemma, respectively. The greater sensitivity of membrane composition to dietary n-6 and n-3 PUFA levels than to SFA and MUFA levels is understandable. The fact that both n-6 and n-3 PUFA classes can not be synthesised *de novo* by higher animals whilst both SFAs and MUFAs can, suggests that the fatty acid composition of membrane lipids will be strongly influenced by the relative abundance of n-6 and n-3 PUFAs in the diet.

Fig. 3 illustrates the influence of the dietary n-3/n-6 ratio on the same ratio in the lipids of the same four cellular membranes (although more commonly expressed in the literature as the inverse n-6/n-3 ratio, hence we have used the n-3/n-6 ratio as diets devoid of n-3 PUFAs would have the mathematically difficult value of infinity). Membrane n-3/n-6 ratio is strongly influenced by diet. In both liver and heart plasma membrane preparations there is an approximately 25% membrane response to 100% variation in diet n-3/n-6 ratio. In synaptosomal membranes there was almost complete (88%) conformity whilst in myelin membrane lipids the change in n-3/n-6 ratio was greater than the dietary change. These results emphasise the importance of the relative balance in n-3 PUFAs and n-6 PUFAs in the diet for membrane lipid composition and the potential importance of this dietary ratio on membrane function.

Fig. 3. The relationship between the ratio of n-3 PUFAs to n-6 PUFAs in the diet and the ratio of these polyunsaturates in the lipids from four membranes (liver plasmalemma, cardiac sarcolemma, cerebral synaptosomes and cerebral myelin) in the rat. Data are from the same sources as Fig. 2. PUFAs, polyunsaturated fatty acids.

The same analysis conducted on the responsiveness of specific membranes to dietary fatty acid composition can be applied to total phospholipid levels of different tissues or to the different phospholipid classes within tissues. Fig. 4 shows the responsiveness to dietary fats of total phospholipid content in six different tissues of the rat. Liver and the large intestine (colon epithelium) phospholipids are the most responsive to dietary changes whilst thymocytes and adipose tissue phospholipids are the least diet-responsive, with skeletal muscle and exocrine pancreas showing an intermediate response. Similar to the membrane preparations, the relative n-6 and n-3 PUFA content of total phospholipids varies more with diet than did SFA and MUFA content.
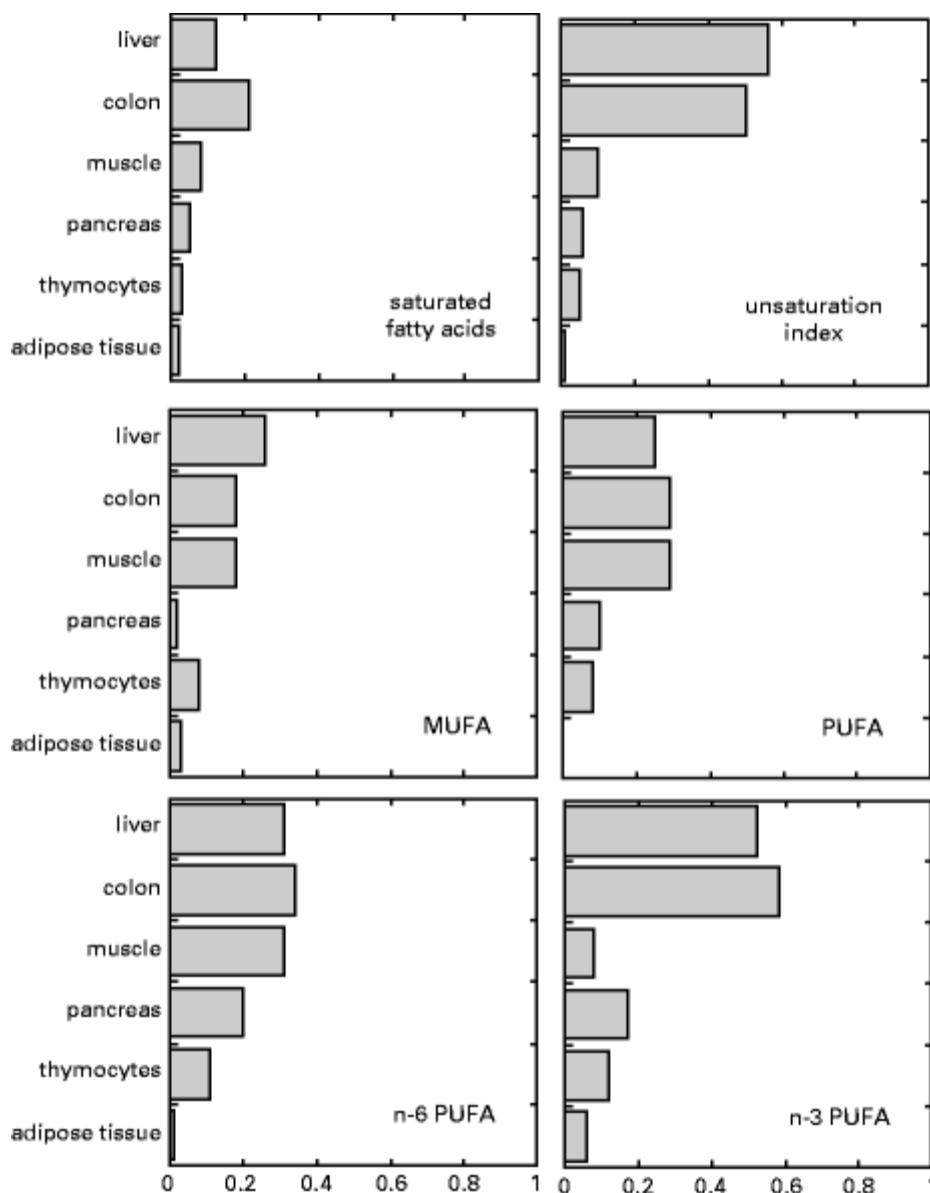
Fig. 4. The responsiveness of phospholipid composition of selected rat tissues to dietary fatty acid composition. A value of 0 represents no relationship whilst a value of 1 represents complete conformity between diet composition and phospholipid composition. Data for this analysis taken from Garg *et al.* (1997); Rock *et al.* (1996); Soriguer *et al.* (2000). MUFA, monounsaturated fatty acid, n-3 and n-6 PUFA, polyunsaturated fatty acids. Unsaturation index is the number of carbon double bonds per 100 fatty acids.

Fig. 5 shows an analysis of different phospholipid classes of the plasma membrane of rat liver cells and their relative responsiveness to variation in dietary fatty acid composition. Both phosphatidylcholine (PC) and sphingomyelin (SPH) (found predominantly in the outer leaflet of the bilayer) were more responsive to variation in dietary MUFA content than the other phospholipid classes. Phosphatidylethanolamine (PE) was more responsive than PC to both dietary n-6 PUFA and the n-3 PUFA docosahexaenoic acid (DHA). SPH was a very diet-responsive phospholipid class, being equally responsive to n-6 PUFA and DHA content in the diet. Both PC and PE were more responsive to dietary DHA than they were to dietary n-6 PUFA. Phosphatidylinositol (PI) was the phospholipid class least responsive to dietary fatty acid variation.
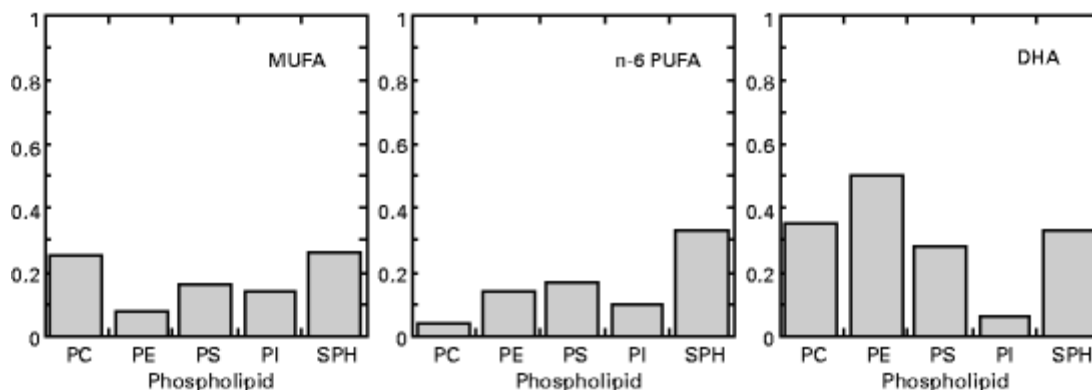
Fig. 5. The responsiveness of different phospholipid classes in the plasma membranes of rat liver cells to aspects of dietary fatty acid composition. A value of 0 represents no relationship whilst a value of 1 represents complete conformity between diet composition and phospholipid composition. DHA=docosahexaenoic acid; PC=phosphatidylcholine; PE=phosphatidylethanolamine; PS=phosphatidylserine; PI=phosphatidylinositol; SPH=sphingomyelin; MUFA, monounsaturated fatty acid; n-6 PUFA, n-6 polyunsaturated fatty acid. Data for this analysis are from Cha & Jones (2000).

These data suggest that membranes are both 'regulator' and 'conformer', that membrane fatty acid composition is predominantly a regulated parameter but that it also partly conforms to (i.e. is strongly influenced by) some aspects of dietary fat composition. The results of the analyses show that although membrane fatty acid composition does change in response to alterations in the fatty acid profile of the diet, these changes are generally much smaller than are the changes in the diet. They show that some classes of phospholipids are more responsive than others and suggest that tissues differ in their relative responsiveness. They suggest a reasonable degree of regulation of the fatty acid composition of membrane bilayers but that the most influential component of dietary fat composition is the relative abundance of n-3 and n-6 PUFAs.

We know relatively little of the mechanisms by which membrane fatty acid composition is regulated however, with information available for only a very few membrane systems. Exactly what membrane variable(s) is/are being regulated is not yet clear. Is it chemical composition *per se* or a particular membrane physical property? It is likely that membrane remodelling is the avenue by which the greatest modification/regulation of membrane composition occurs and this appears to result from rapid deacylation/reacylation processes. The enzymes responsible for the regulation of membrane fatty acid composition include elongases, desaturases, phospholipases and lysophospholipid acyltransferases. As will be described in the next section, membrane fatty acid composition differs between species in a systematic manner. It is likely that the genome specifies membrane composition of different species by specification of these enzymes and that genetic influences on the membrane composition of individuals also is mediated *via* effects on these membrane-bound enzyme systems.

It has long been known that membrane viscosity is generally maintained in a self-regulating homeostatic manner and that this involves desaturases (Kates, Pugh & Ferrante, 1984). Similarly, the affinity of a key enzyme involved in membrane remodelling, microsomal acyltransferase, for its acyl-CoA substrates is strongly influenced by its surrounding lipid environment (Fyrst *et al*., 1996). This self-regulation of membrane composition is compromised by the fact that both n-6 and n-3 PUFAs are unable to be synthesised *de novo* by mammals and thus the polyunsaturate content of the diet has a greater influence on membrane composition and consequently on membrane function and metabolism than do either saturated or monounsaturated dietary fats.

5. DIETARY FATS, MEMBRANE FUNCTION AND ENERGY METABOLISM

Several studies have shown that changing the fatty acid profile of the diet alters energy expenditure. Rats fed n-6 PUFAs had lower body fat content and an increased metabolic rate compared to those fed a saturated fat diet (Shimomura, Tamura & Suzuki, 1990). Pan and Storlien (1993) reported that metabolic rate was elevated in rats fed an n-3 PUFA-enriched diet, compared with rats consuming diets containing saturated fats alone. In an extensive study of rats fed isocaloric diets containing either saturated fat, MUFA, n-6 PUFA, or n-3 PUFA, the animals fed the saturated fat diet had the lowest metabolic rate, with the greatest weight gain and the highest body fat content (Takeuchi *et al*., 1995). These findings, along with those on baboons (Savage & Goldstone, 1965) and birds (Newman *et al*., 2002), indicate that increasing the PUFA content of the diet increases metabolic rate.

While fewer investigations have been conducted with humans the trends appear to be similar. Jones and Schoeller (1988) compared subjects consuming diets differing in their polyunsaturated to saturated fat ratio and consistent with the animal studies, the more polyunsaturated diet was associated with a greater metabolic rate. Similarly, in another human study the more polyunsaturated diet was associated with an increased resting metabolic rate and also a greater thermogenic response to food (Van Marken Lichtenbelt, Mensink & Westerterp, 1997). Couet *et al*. (1997) conducted a human crossover study in which the polyunsaturated to saturated fat ratio of the two diets was identical (0.2), however the diets differed in the type of PUFA they contained. Consumption of the diet enriched with fish oil (high in n-3 PUFAs) was associated with a reduced body fat mass and increased resting metabolic rate, suggesting that long chain n-3 PUFAs are also effective in stimulating the metabolic rate of humans.

Taken collectively these studies indicate that increasing the level of PUFA in the diet elevates metabolic rate and although none measured dietary-induced changes in membrane composition, this avenue is likely to be the mode of action of enhanced dietary PUFA. This conclusion is heavily influenced by recent studies explaining the metabolic rate of different species and particularly the development of what has been called the 'membrane pacemaker' theory of metabolism (Hulbert & Else, 1999, 2000).

It has become obvious that a large part of the cost of living and thus of an animal's metabolism is due to membrane-associated processes. The contribution of various energy-consuming processes to the resting metabolic rate of mammals has been reviewed (Rolfe & Brown, 1997). Approximately 70% of resting metabolism is due to mitochondrial ATP synthesis, while about 20% is due to the mitochondrial proton leak and approximately 10% of mammalian resting metabolic rate is non-mitochondrial oxygen consumption. The ATP produced is consumed as follows: approximately 28% is used by protein synthesis, 19–28% by the Na^+/K^+-ATPase, 4–8% by the Ca^{2+}-ATPase, 2–8% by actinomyosin ATPase, 7–10% by gluconeogenesis, 3% by ureagenesis, with other processes including mRNA synthesis and substrate cycling consuming the remainder. While this relative breakdown of resting metabolic rate is largely derived from data for the rat, it seems to be more general. For example, liver cells from mammals ranging from mice to horses have different respiration rates but almost identical relative contributions from mitochondrial ATP production, mitochondrial proton leak and non-mitochondrial oxygen consumption (Porter & Brand, 1995). Similarly, the Na^+/K^+ pump accounts for about the same proportion of liver metabolism in mammals, reptiles, fish, amphibians and birds (Else & Hulbert, 1987; Hulbert & Else, 1990).

Mass-specific resting metabolic rate varies dramatically (>100-fold) between different animals and a consistent finding has been that species with high metabolic rates have highly polyunsaturated membranes while species with low metabolic rates have cellular membranes

that are more monounsaturated (see Hulbert & Else, 1999, 2000). A particularly important fatty acid in this respect is the long chain n-3 PUFA, docosahexaenoic acid. The relative concentration of 22:6 n-3 in tissue phospholipids shows the greatest correlation with body size (and consequently metabolic rate) in both mammals (Hulbert, Rana & Couture, 2002*b*) and birds (Hulbert et al., 2002*a*). A comparison of the Na^+/K^+-ATPase enzyme in tissues from 13 species of bird and mammal showed that large variation (>18-fold) in the molecular activity (rate of substrate turnover, in this case ATP per enzyme molecule per minute) of this membrane-bound enzyme was strongly correlated with the 22:6 n-3 content of the surrounding phospholipids (Turner, Hulbert & Else, 2003). Evidence that this correlation is likely to be causal is supplied by a series of 'species membrane-crossover' experiments which show that Na^+/K^+ pump molecular activity was dependent upon the acyl composition of the membrane into which it was reconstituted (Else & Wu, 1999; Wu *et al*., 2004). There is also evidence that the stimulatory effect of this highly polyunsaturated fatty acid may be mediated *via* the physical properties of the membrane surrounding the Na^+/K^+-ATPase enzyme (Wu *et al*., 2001).

Another membrane-associated process quantitatively important in metabolism is the mitochondrial proton leak and it too has been reported to be correlated with mitochondrial 22:6 n-3 content (e.g. Brookes *et al*., 1998). A causal link in this correlation is supported by both dietary and lipid fusion experiments where liver mitochondria of mice increase their proton leak when membrane 22:6 n-3 content was increased (Stillwell *et al*., 1997).

Polyunsaturated fatty acids are also associated with many other rapid membrane-linked processes. For example, retinal membranes have high 22:6 n-3 content and this is associated with high activity of visual system G-proteins (Litman & Mitchell, 1996). Furthermore the Ca^{2+}-ATPase, which is also a significant component of metabolism, particularly in muscles, has been found to be highly active in membranes containing high levels of PUFA, especially 22:6 n-3 (Infante, 1987; Infante, Kirwani & Brenna, 2001).

In summary, the significant, if relatively small effects that dietary fat composition has on metabolic rate are probably mediated by the limited effects such dietary composition changes have on the fatty acid composition of membrane lipids. Polyunsaturated fats, and especially n-3 PUFAs increase metabolic rates of mammals. Such an effect is compatible with the 'membrane pacemaker' theory of metabolism and the very large differences in membrane fatty acid composition between different higher vertebrates that are associated with very large differences in the metabolic rates of these species.

An interesting implication of these findings is that the different metabolic rates of particular species that have been previously associated with their specific food habits may have a mechanistic explanation rather than being the result of selective pressures as has been previously argued (e.g. see McNab, 2002). For example, sea birds have basal metabolic rates that are higher than expected for their body size and this may be due to a fish diet with its high n-3 PUFA content. Similarly, it is tempting to speculate that the lower-than-expected metabolic rate of termite-eaters is due to a diet with a low PUFA content.

We have concentrated, in this section, on the effects of dietary fat composition on the metabolic rate of animals, presumably mediated by dietary-induced changes in membrane fatty acid composition. However, modification of dietary fat composition has been shown to have many other effects on the physiology and biochemistry of animals. For example, dietary n-3 PUFAs affect the 'sickness behaviour' (or 'acute phase response') of mice during inflammation (Kozak *et al*., 1997). The exercise endurance of rats differs greatly depending whether they are fed diets high in n-6 or n-3 PUFAs (Ayre & Hulbert, 1997). The PUFA content of the diet significantly influences the preferred body temperature selected by reptiles (Geiser & Learmonth, 1994) and the arousal behaviour of hibernating mammals (Geiser & Heldmaier, 1995). These diverse findings illustrate a few examples of the great variety of

effects that have been reported as a result of modifying the fat composition of the diet of animals. In most cases, the *modus operandi* of such effects are unknown but many are possibly the result of diet-induced changes in membrane fatty acid composition. In the following section we focus on two major disease areas of enormous impact on human health: the metabolic syndrome and mental illness.

6. DIETARY FATS, MEMBRANES AND DISEASE

If membrane fatty acid composition is largely a regulated phenomenon and membrane lipid composition is most responsive to the PUFA composition of the diet, then current Western diets present a challenge to modern humans. Until recently the dietary balance between n-3 and n-6 PUFAs has been evenly balanced with a n-3/n-6 ratio of approximately 1; the modern Western diet is now dominated by n-6 PUFA, with a n-3/n-6 ratio of approximately 0.06 (Simopoulos, 2002). While the regulatory mechanisms that maintain the balance of our membrane composition might provide some protection from this major shift in dietary fat consumption the ability of membrane lipid composition to remain completely unaffected is unlikely. This is shown in Table 2 for the membrane phospholipid composition for brain of rats fed diets extremely different in fat composition and, given the conundrum of the modern era where enhanced access to food is associated with major changes in both the amount and quality of dietary fat, the implications of this are broad-reaching. Two broad disease areas, the metabolic syndrome and mental illness will now briefly be discussed in this context.

6.1   Metabolic Syndrome

The term 'metabolic syndrome' was developed to describe a cluster of disease states that include blood lipid disorders, hypertension, propensity for thrombus formation, low-grade chronic inflammation, abdominal obesity, and type 2 diabetes (for a recent review see Grundy et al., 2004). The metabolic syndrome is common in much of the developed world. For example, it is present in more than 23% of the US adult population and is associated with several modifiable lifestyle factors (Park et al., 2003). The key to understanding the metabolic syndrome is insensitivity to insulin and particularly the relative failure of insulin to exert its multiple biological effects on carbohydrate and lipid metabolism. Fat balance is a central concern with the focus usually on dietary fats as an energy source. However, the roles of fats as major components of membranes and as potent metabolic intermediates in cellular signalling are equally important in expression of the metabolic syndrome disease cluster. The conclusions from animal and human studies is that the insulin-resistance-inducing and obesogenic properties of fats can be laid at the single-bonded feet of saturated fats, while a case can be made for PUFAs being protective (Storlien, Hulbert & Else, 1998; Vessby, 2000; Vessby et al., 2001, 2002).
Insulin-resistance is an underlying factor fundamental to the initiation of this disease syndrome. For example, when rats are placed on a high-fat, refined-sugar diet they first become insulin-resistant and hyperinsulinemic (within two weeks, the earliest sampling period) and later obesity begins (by two months, the next sampling period) followed at twelve months by hypertension (Barnard et al., 1998). In humans, whilst genetic predisposition plays a significant role, and lifestyle factors including diet, are important (Park et al., 2003), the fat quality of the diet also clearly unmasks the phenotype.

The fatty acid composition of membrane lipids is influenced (see above) by the dietary fatty acid profile and there is now considerable evidence linking obesity and insulin resistance to

the fatty acid composition of membrane phospholipids (Borkman et al., 1993; Vessby, Tengblad & Lithell, 1994; Pan et al., 1995). Most of these experimental studies have been carried out in skeletal muscle, the major tissue for insulin-stimulated glucose disposal. They show that a decreased level of unsaturation of skeletal muscle phospholipids relates directly to impaired insulin action, and to various measures of increased regional and total adiposity. Conversely, PUFAs in phospholipids, and particularly the highly unsaturated n-3 PUFA class, convey protection against both insulin-resistance and development of obesity and type 2 diabetes.

There are two proposed mechanisms to underpin such observations. The first is through effects on metabolic activity. Decreased membrane polyunsaturation may act to decrease the activity of the major energy-consuming processes of the cell such as reducing the flux of ions and protons and subsequently the energy needed to maintain ionic homeostasis. Since such processes contribute heavily to the basal metabolism of the cell, decreased polyunsaturation of membranes will thus decrease metabolic rate. This, in turn, will predispose to an increased accumulation of body fat stores for any given level of nutrient intake (i.e. a positive fat balance). Of course such positive fat balance will result in whole-body fat accretion as well as accumulation in the liver and skeletal muscle, both critical for insulin action. The literature showing increased intramyocellular lipid closely associated with muscle insulin resistance is now large and convincing (see Kelley, Goodpaster & Storlien, 2002). Conversely, more n-3 polyunsaturated fats will increase metabolic rate and thus impact favourably on fat balance. Interestingly, increased membrane unsaturation provides an environment conducive for the enhanced intrinsic activity of ion transporters thus providing the conditions, in concert, to allow maintenance of ion homeostasis (Wu et al., 2004).

A second mechanism that would allow membrane fatty acid composition to influence insulin action is via alteration of membrane proteins specifically associated with the action of insulin. Such mechanisms would include modulation both of receptor affinities and of translocation to the membrane of nutrient transporters (as well as possible enhancement of the intrinsic activity of these nutrient transporters). Changes in the affinity of the insulin receptor were demonstrated in early in vitro studies which showed impaired insulin binding when saturated fatty acids were added to the medium (Grunfeld, Blair & Kahn, 1981; Field et al., 1988). Conversely there is evidence that highly unsaturated n-3 PUFAs are beneficial to insulin receptor function (Sohal, Baracos & Clandinin, 1992; Clandinin et al., 1993). It is interesting in this context that insulin sensitizers such as bezafibrate (Matsui et al., 1997) are known also to increase the unsaturation of membrane lipids, and part of their beneficial action may be a membrane-lipid-mediated effect on the insulin receptor. Dietary treatment emphasizing saturated fat intake is reported to decrease β-adrenergic receptor affinity (Matsuo, Sumida & Suzuki, 1995), an observation consistent with decreased metabolic rate. There is also evidence now emerging that diets high in saturated fats act to increase neuronal activity in brain hypothalamic areas associated with feeding and suppress activity in areas associated with satiety and increased energy expenditure (Wang, Storlien & Huang, 1999). The possibility that these responses come about via diet-influenced membrane lipid changes modulating membrane receptors should be explored.

In addition to the lipid-protein interactions occurring within membranes linking membrane lipid composition to some key metabolic derangements of the metabolic syndrome, membrane lipids may influence intracellular lipid and carbohydrate metabolism more directly. These mechanisms include contributing metabolic products that are themselves involved, or by generating second messenger molecules which modulate activities of key enzymes This area has been recently reviewed (see Kelley, Goodpaster & Storlien, 2002). In summary, the relationship between diet and fatty acid profile of membrane bilayers may be a

key that can open new doors in the battle against the current major epidemic of type 2 diabetes and obesity.

6.2   Mental Illness

The relationship between food, mood and behaviour is well established (Christensen, 2001), and an important role for dietary fats in mental illness is becoming apparent. Large-scale population studies have shown persistent negative correlations between fish consumption (high n-3 PUFA consumption) and depression (Hibbeln, 1998; Tanskanen, 2001; Tanskanen et al., 2001; Silvers & Scott, 2002). Low n-3 PUFA levels and low n-3/n-6 ratios in plasma have been associated with depressive disorders (Tiemeier et al., 2003) and numerous studies have reported lower levels of n-3 and n-6 PUFAs in membrane phospholipids of people suffering depression and schizophrenia (see Table 1 in Fenton, Hibbeln & Knable, 1999). These deficiencies in PUFAs have been primarily observed in erythrocyte membranes (Peet et al., 1998) although adipose tissue of depressed subjects (Mamalakis, Tornaritis & Kafatos, 2002) as well as phospholipids of postmortem brain samples of schizophrenics (Yao, Leonard & Reddy, 2000) shown similar results. An example of the differences found in the phospholipid fatty acid composition of postmortem brain from a control group and schizophrenics is shown in Table 2 (data supplied by X.F. Huang and W. Bell, personal communication).

Intervention studies using dietary PUFAs show benefits in some but not all studies. Reduction in stress-induced aggression (Hamazaki et al., 1996) and mood-stabilising effects in bipolar disorder (Stoll et al., 1999) have been reported with increased DHA intake. Dietary eicosapentaenoic acid supplementation (EPA; 20:5 n-3) has also been shown to be effective in treating depression (Peet & Horrobin, 2002). However, other studies on major depression found no significant effect following DHA (Marangell et al., 2003) or EPA supplementation (Fenton et al., 2001). Animal studies have shown that the reversal of DHA deficiency in adult animals is particularly slow in brain and retina (approximately 8 weeks) compared to serum and other tissues (approximately 2 weeks; Moriguchi et al., 2001) whereas foetal brain accumulates DHA rapidly following deficit (within days; Schiefermeier & Yavin, 2002). This suggests long-term interventions (even when serum levels are raised) may be required to detect significant psychotropic effects following DHA supplementation in adults. Interestingly, the Marangell et al. (2003) study, found no effect of DHA upon major depression following six weeks of supplementation whereas the studies of Peet and Horrobin (2002) and Stoll et al. (1999), that demonstrated improvement, used EPA and DHA supplementation for 12 and 16 weeks, respectively.

A potential area to test the relationship between dietary PUFAs, membrane lipid composition and depression is in the postnatal depression that affects approximately 15% of pregnancies (Pope et al., 2000). Postnatal depression is often more severe and common during pregnancy than after childbirth, with 8 weeks before birth being a high-risk period (Evans et al., 2001). During this period, the foetus is undergoing unprecedented growth including a gross increase in the size of the brain. This will require the mother to mobilise essential fatty acids. A longitudinal study of pregnant women has shown that total phospholipid plasma fatty acid levels increase by approximately 50%, with arachidonic acid (AA) and DHA increasing by 23% and 52%, respectively, during this period (Al, Van Houwelingen & Hornstra, 2000).

Foetal demand for DHA in the third trimester is estimated to be approximately 67 mg day^{-1} whereas maternal intake of EPA and DHA in Canadian women has shown intakes ranging from 4 to 125 mg day^{-1} and 24 to 524 mg day^{-1}, respectively. This study clearly indicates inadequate DHA intake in some pregnant women (Innis & Elias, 2003) and the possibility is that long-chain PUFAs may alternatively be derived from the mobilisation of maternal stores

in these cases. A study of Korean women showed that pregnancy significantly decreased red blood cell membrane DHA levels (Ghebremeskel et al., 2000). Other maternal tissues, including the membranes of the brain (with up to 13% of fatty acids in grey matter as DHA: see Table 2) are other sources that could service this demand. The consequence of a foetal brain drain on maternal PUFA levels could manifest itself in maternal depression. Women treated with EPA and DHA (to avoid the potential harmful effects of conventional antidepressants) have shown consistent reductions in depression scores following treatment (Chiu et al., 2003). Postnatal supply of PUFA, through breast-feeding, could continue any drain on maternal supplies, an idea supported by a significant inverse correlation between the DHA content of mothers milk and the prevalence of postpartum depression (Hibbeln, 2002).

Table 2: Comparison of major fatty acids of phospholipids from human (schizophrenia and control) and rat (following extreme dietary fat manipulation) cingulate cortex brain region

| | Human ($N=6$) | | | | Rat ($N=4-6$) | | | |
| | Grey matter | | White matter | | | | | |
Fatty acids %	Schizo-phrenia	Control	Schizo-phrenia	Control	Low fat	High SAT fat	n-6 PUFA	n-3 PUFA
16:0	15.8[⑤]	18.0	11.7	12.8	34.5	26.5	26.0	26.8
18:0	19.2	20.3	19.3	18.6	18.9	22.0	21.3	21.6
18:1 n-9	29.3[⑤]	25.3	36.3	34.5	18.3	19.8	16.6	17.7
20:4 n-6	8.2	9.5	4.2	4.0	10.2	11.0	11.6	8.1*
22:4 n-6	8.1	7.9	6.6	6.8	6.5	4.2	6.5	1.1*
22:6 n-3	10.4[⑤]	13.1	3.4	2.3	10.6	13.8	10.9	19.1*
% SATs	38.8	41.0	34.5[⑤]	36.4	52.4	49.1	47.7	49.1
% MUFAs	33.3	28.5	46.6	44.2	20.3	21.7	21.7	22.1
% PUFAs	27.9[⑤]	31.6	16.4	14.8	27.2	29.2	30.5	28.9

All data kindly provided by Mr. W. Bell and Dr. X. F. Huang (personal communication). All fatty acid values are expressed as relative percentage where only fatty acids >4% of total reported. *Postmortem* human brain samples provided by NISAD (Neuroscience Institute of Schizophrenia and Allied Disorders). Percentage and source of energy content of rat dietary groups following 12 weeks of feeding: low fat (2%, safflower oil); high sat fat (35%, beef tallow, 2%, safflower oil); n-6 PUFA (38%, safflower oil); and n-3 PUFA (35% fish oil, 2%, safflower oil). Male to female ratio 5:1. Significant difference between groups at the $P<0.05$ signified by [⑤] for comparisons between schizophrenic and control human samples (Student T-test) and by * for comparisons between rat dietary groups (Anova-Tukey *post hoc* test). SATs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids.

An alternative interpretation of the low PUFA levels in erythrocyte membranes of schizophrenics and people with schizo-affective disorders is suggested in a recent study by Hibbeln et al. (2003). This study proposes that reduced PUFA levels in red blood cell membranes is more related to smoking and gender than to the state of mental health. Smoking is commonly associated with depression and other psychiatric disorders (Ziedonis & Williams, 2003). Most schizophrenics smoke and this can have a major effect on erythroctye DHA and EPA levels, particularly in women. Hibbeln et al. (2003) found that non-smoking females showed significantly higher EPA (31%) and DHA (42%) levels compared to non-smoking males. Smoking produced no significant effect upon EPA or DHA levels in males, but large reductions for females, enough to produce a significant difference for smokers versus non-smokers combined (even though women represented only 37% of the smokers in the study). Non-smoking females also reported higher dietary intake of n-3 in the form of linolenic acid (LNA) compared to males and other females. Whether smoking also influences membrane PUFA levels of other tissues such as the brain, is unknown. The post-mortem study of Yao et al. (2000), that showed PUFA changes in the brain of schizophrenics, had matched numbers of smokers in the control and schizophrenic groups which suggests that these changes may not be due to smoking. As pointed out by Hibbeln et al. (2003), future

studies need to control for numerous variables, including smoking, in order to test the proposition that PUFA levels are related to mental health.

Deficits in membrane polyunsaturation can occur for a number of reasons. These include inadequate dietary supply, biochemical dysfunction and PUFA oxidation. In dietary deficiency of n-3 PUFA (but not n-6 PUFA) normally there is a trend for reciprocal replacement of 22:6 n-3 with the n-6 PUFA near-equivalents, i.e. 22:5 n-6 and to a lesser extent 22:4 n-6 (Neuringer, Andersen & Connor, 1988; Carrie et al., 2000; Murthy et al., 2002). In the case of depression and schizophrenia, this reciprocal replacement does not occur, with both n-6 and n-3 PUFAs reduced overall, and MUFAs and SFAs making up the difference (see Peet et al., 1998). This pattern might be indicative of oxidative damage and/or biochemical dysfunction or a general PUFA dietary deficiency rather than a specific n-3 PUFA deficiency. In summary, membrane lipid composition appears to influence the state of mental health and is likely to exert its effects through lipid-protein interactions within the membrane similar to those described for the metabolic syndrome. Such interactions may include effects upon neurotransmitter release and reuptake, and membrane receptors.

7. A PERSPECTIVE ON THE MODERN HUMAN DIET

Modern humans are unique in that the types of food eaten can vary dramatically between individual humans within a population (unlike wild animals, where although the amount of food might vary, the fatty acid profile of the diet is likely to be relatively constant because of the relative constancy of the particular type of food they consume). For individual humans the consequence of this variability in the types of food eaten is that the dietary fatty acid profile is similarly highly variable. The health consequences of variability in the fatty acid profile between individual humans within a population may now be emerging as discussed in the importance of dietary PUFAs in the metabolic syndrome and the emerging link with mental health.

The reciprocity of n-3 and n-6 PUFAs and their importance for membrane function and relative abundance of these two types of PUFA in the human food chain may have as yet generally unappreciated health consequences. An evaluation of the quantitative changes of the availability of fats in the US food supply from 1909 to 1999 (Hibbeln, Lands & Lamoreaux, 2002) has provided some fascinating but alarming results. From 1909 to 1999, the total availability of PUFA in the US increased from 11.7 g person^{-1} day^{-1} to 34.3 g person^{-1} day^{-1}. This increase was almost solely due to an increase in soybean oil which increased from 0.3 to 31.2 g person^{-1} day^{-1} for the 1909–1999 period. As pointed out earlier in this contribution, soybean oil is overwhelmingly a n-6 PUFA dominated oil with a n-3/n-6 ratio of 0.1 (Beare-Rogers, 1989) and thus implies the US food chain has become dominated by linoleic acid during the last century. For the same period the availability of long-chain n-3 PUFA only increased from 0.2 to 0.3 g person^{-1} day^{-1}. This is potentially a dire situation, worthy of urgent investigation in light of the emerging importance of membrane fatty acid composition (especially n-3 PUFA) in human health. The previous adequate intake of n-3 PUFA in green leaf food sources may be increasingly overwhelmed by the dominance of n-6 PUFA in manufactured foods. The increased consumption of manufactured foods, when coupled with the overwhelming influence of soybean oil as a fat source in the US food chain, may turn out to be a dramatic unforeseen consequence of monoculture in agriculture. Such a situation is probably not restricted to the US but may be a more general phenomenon. While 'you are what you eat' may not be perfectly true, there is no doubt that the expanded 'you are influenced by what you eat' is true.

8. CONCLUSIONS

The n-6 and n-3 PUFAS are important constituents of cellular and sub-cellular membranes, they are also unable to be synthesised by vertebrates and therefore must be obtained preformed as essential components of the diet. As a consequence their relative abundance in the diet has a major influence on the composition of membrane bilayers.

Membrane composition, especially the level of polyunsaturation of membranes plays a role in determining the metabolic rate of cells and consequently of the whole animal – as detailed in the 'membrane pacemaker theory' of metabolism. This theory also provides perspective to the previously reported associations between the distinctive metabolic rates of species with particular food habits. The theory would propose that differences in metabolic rate between species may not be due to evolutionary adaptation to particular foods but may instead have a more mechanistic explanation related to the fatty acid profile of the particular food types. For example, sea birds have higher, and termite-eaters lower, than expected metabolic rates because to some extent 'they are what they eat'.

Substantial changes in dietary fatty acid composition are often required for relatively small changes in membrane composition. This is due to the regulation of membrane fatty acid composition. The precise mechanisms and the property or properties being regulated are not yet clearly understood.

Tissues vary in their responsiveness to changes in dietary fat composition, as do different sub-cellular membranes and different membrane lipid classes. Membrane composition is most responsive to the n-6 and n-3 PUFA levels in the diet and especially to the n-3/n-6 ratio of dietary PUFAs. This is probably because these two different types of PUFA are often able to substitute for each other.

Membrane functions are influenced by dietary-induced changes in membrane lipid composition.

Modern humans have over the last century emphasised n-6 PUFAs at the expense of n-3 PUFAs. The health consequences of this change are only starting to be appreciated.

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