Influence of dietary fat on the lipid composition of rat brain synaptosomal and microsomal membranes

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The modulation of rat brain microsomal and synaptosomal membrane lipid by diet fat was examined. Brain synaptosomal and microsomal membrane composition was compared for rats fed on diets containing either soya-bean oil (SBO), SBO plus choline, SBO lecithin, sunflower oil (SFO), chow or low-erucic acid rape-seed oil (LER) for 24 days. Cholesterol and phosphatidylcholine levels in both membranes were altered by diet. Diet fat also affected the microsomal content of sphingomyelin. Change in membrane phosphatidylcholine level was related to the relative balance of $\omega-6$, $\omega-3$ and monounsaturated fatty acids within the diets fed. The highest phosphatidylcholine levels appeared in membranes of animals fed on SBO lecithin and the lowest in those fed on LER. Microsomal membrane cholesterol and sphingomyelin content increased by feeding on SBO lecithin. In both synaptosomal and microsomal membranes a highly significant correlation was observed between membrane phosphatidylcholine and cholesterol content. The fatty acyl composition of phospholipids from both membranes also altered with diet and age. Alteration in fatty acid composition was observed in response to dietary levels of $\omega-6$, $\omega-3$ and monounsaturated fatty acids, but the unsaturation index of each phospholipid remained constant for all diet treatments. These changes in lipid composition suggest that dietary fat may be a significant modulator *in vivo* of the physicochemical properties of brain synaptosomal and microsomal membranes.

The fluid mosaic model of membrane structure (Singer & Nicholson, 1972) emphasizes the dynamic nature of membrane lipids and proteins. The influence of membrane lipid composition on the physicochemical properties of the membrane has been well documented (Thilo *et al.*, 1977; McMurchie & Raison, 1979). Alterations in physical properties of membrane lipid could be mediated through changes in phospholipid fatty acids, phospholipid polar head groups or membrane cholesterol content (Chapman, 1975; Boggs, 1980). Such changes in membrane lipid composition have been obtained experimentally by alteration of environmental temperature in poikilotherms (Wodtke, 1981) and bacteria (Yang & Haug, 1979), or in homoeotherms by feeding different dietary fats (Innis & Clandinin, 1981a,b; King *et al.*, 1977; Ehrstrom *et al.*, 1981). Mammalian dietary studies have attempted to change the lipid composition of brain

membranes (Matheson *et al.*, 1981; Selivonchick & Roots, 1979; Koblin *et al.*, 1980). However, comparisons made in the majority of these studies are between essential fatty acid-deficient diets and diets high in $\omega-6$ and $\omega-3$ fatty acids. (These latter terms indicate the position and spacing of the double bond in unsaturated fatty acids, with ω representing the terminal-methyl-group locant.) Changes were observed in membrane lipid composition and in some cases these alterations could be correlated with altered membrane function, e.g. ($\text{Na}^+ + \text{K}^+$)-dependent ATPase activity (Sun & Sun, 1974), anaesthetic requirement (Koblin *et al.*, 1980) and ethanol tolerance (John *et al.*, 1980).

The present study was undertaken to determine the effect of nutritionally adequate diets containing various dietary fats on the polar lipid composition of rat synaptosomal and microsomal membranes. Soya-bean lecithin was fed as it has been suggested that dietary phospholipids may alter the phospholipid composition of brain (Heger, 1979). Diets

Abbreviations used: SBO, soya-bean oil; SFO, sunflower oil; LER, low-erucic acid rape-seed oil.

high in lecithin have been shown to raise brain choline and acetylcholine levels (Cohen & Wurtman, 1976; Magil *et al.*, 1981). If lecithin diets could also result in changes in brain membrane lipid composition such dietary treatments could have significant implications for the function of membrane-dependent enzymes in the brain.

Materials and methods

Animals and diets

Male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Ontario, Canada) weighing 54.6 ± 3.81 g were fed on diets containing 20% (w/w) fat as SBO (Crisco liquid shortening), soya-bean lecithin (Monarch Fine Foods, Toronto, Ontario, Canada), SFO (Unico Foods, Toronto, Ontario, Canada) or LER (CSP Foods, Altona, Manitoba, Canada). In addition, a group of rats were fed on a diet containing SBO plus choline, equivalent to the choline level supplied by the lecithin diet. A sixth group of rats was fed on laboratory stock chow.

The basal diet utilized contained the following nutrients per kg of diet: 270 g of high-protein casein; 200 g of starch; 207.65 g of glucose; 200 g of fatty acid; 50 g of non-nutritive cellulose; 10 g of vitamin mix; 50.85 g of mineral mix; 2.75 g of choline; 6.25 g of inositol and 2.5 g of L-methionine. The composition of the vitamin mix (Teklad Test Diets, Madison, WI, U.S.A.) and Berhart-Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH, U.S.A.) have been described (Innis & Clandinin, 1981a). The fatty acid composition of each dietary fat is illustrated (Table 1). SBO lecithin was not pure phosphatidylcholine and in addition to phospholipid (84%, w/w) contained small quantities

of non-esterified fatty acids (1.4%, w/w), triacylglycerol (14%, w/w) and cholesterol (<0.6%, w/w). Diet treatments resulted in similar rates of growth except for those animals fed SBO plus choline (Table 2). Decreased growth response was observed for animals fed on the latter diet.

Rats were housed individually in a temperature-controlled room at $21 \pm 1^\circ\text{C}$. Diets and water were supplied *ad libitum*. Three replicates of four rats in each diet treatment were fed for 24 days and then killed by decapitation.

Membrane isolation and lipid analysis

Synaptosomal and microsomal membranes were isolated from rat brain by preparative ultracentrifugation as described previously (Cruz & Gurd, 1978). Membrane purity obtained by these procedures has been characterized previously (Cruz & Gurd, 1978). Synaptosomal and microsomal lipids were extracted and quantitatively analysed for phospholipid and cholesterol content utilizing a flame-ionization detector (Foot & Clandinin, 1982). Phospholipids were purified from the remaining total lipid extract by separation from neutral lipids on Analtech silica gel H T.L.C. plates ($20\text{ cm} \times 20\text{ cm}$) obtained from Mandel Scientific Co. (Rockwood, Ontario, Canada) with light petroleum (b.p. $50\text{--}110^\circ\text{C}$)/diethyl ether/acetic acid (85:15:1, by vol.) as the solvent system. Individual phospholipids were separated in two dimensions on silica gel H T.L.C. plates ($20\text{ cm} \times 20\text{ cm}$) (Innis & Clandinin, 1981a). Phosphatidylcholine and phosphatidylethanolamine spots were converted into methyl esters by using BF_3 (Morrison & Smith, 1961).

Fatty acid methyl esters were separated by automated glass capillary G.L.C. (model 3700 gas

Table 1. *Fatty acid composition of diets*

Diets contained 20% (w/w) of fatty acid with the exception of the laboratory stock chow. The oils utilized were of fully processed edible quality. The SBO plus choline diet contained an additional 32 g of choline/kg of diet to raise the choline content of this diet to be equivalent to that of the lecithin diet.

Fatty acid	Fatty acid (% w/w)				
	SBO*	SBO lecithin	SFO	Chow	LER
C _{14:0}	0.08	0.06	0.1	2.78	0.03
C _{16:0}	9.65	16.15	7.07	24.30	4.57
C _{16:1} (ω -7)	0.09	0.08		5.94	0.08
C _{18:0}	3.27	4.01	3.09	4.68	1.21
C _{18:1} (ω -9)	36.97	12.50	13.44	27.30	57.90
C _{18:2} (ω -6)	43.1	55.32	75.89	31.99	23.66
C _{18:3} (ω -3)	4.44	9.03	0.32	3.01	9.22
C _{20:0}	0.2	0.04			1.02
C _{20:1} (ω -9)	0.30				1.01
C _{22:1} (ω -9)					0.17
C _{24:0}					0.1

* SBO also contained minor amounts of positional and geometric isomers of C_{18:1}.

chromatograph and model 8000 auto sampler; Varian Canada, Georgetown, Ontario, Canada) and quantified using flame-ionization detectors. Chromatography was performed utilizing an open tubular glass capillary column (18 m $\times$ 0.25 mm internal diameter) coated with SP1000 (grade AA column supplied by Supelco Inc.) as previously described (Innis & Clandinin, 1981a). Fatty acid methyl esters were identified and weight percentage fatty acid composition was computed by a chromatography data system (CDS III, Varian Canada) as described previously (Innis & Clandinin, 1981a).

Statistical analysis

The effect of dietary treatment was examined by multivariate analysis of variance and covariance techniques. Comparison between individual diets was made by using Neuman-Keuls multiple-range test after an effect of diet treatment was shown by analysis of variance.

Results and discussion

Lipid composition of brain membranes

Synaptosomal membranes. The phospholipid and cholesterol content of rat brain synaptosomal membrane was determined (Table 2). The phospholipid/protein ratio was 0.54–0.58 mg of phospholipid/mg of protein, some 0.14 mg lower than previously reported for rat brain synaptosomes (Breckenridge *et al.*, 1972; Cotman *et al.*, 1969), but similar to values reported by Lapetina *et al.* (1968) for rats, and by Chin *et al.* (1978) for mice. The lower phospholipid content observed could be due to omission of minor phospholipid constituents such as

phosphatidylserine, phosphatidylinositol and lyso-phosphatidylcholine not accurately measured by the chromarod/FID method of analysis and hence excluded from the summarized data (Table 2). Synaptosomal cholesterol content was 0.18 mg of cholesterol/mg of protein at day 0, which is within the range reported for rodent brain (Breckenridge *et al.*, 1972; Chin *et al.*, 1978). Ethanolamine glycerophospholipid content of the synaptosomal membrane is 50% of total phospholipid, higher than observed by Cotman *et al.* (1969) and Chapman (1972) but similar to that reported by Whittaker (1969). The major phospholipid is phosphatidylcholine. The ethanolamine plasmalogen content of the ethanolamine glycerophospholipids fraction was less than 50% of the total (Table 2). Differences between previously reported observations for brain polar lipid content and those of the present study could be due in part to differences in the age of animals examined. Most brain lipid analyses reported previously are for membranes from adult rats versus those from weanling rats used in the present study. Small changes in phospholipid composition suggestive of developmental changes were observed independent of diet treatment (Tables 2 and 3). Analogous developmental changes in polar-head-group composition, cholesterol content and fatty acyl group compositions have been observed for rat brain endothelial cells (Matheson *et al.*, 1980), whole rat brain (Alling *et al.*, 1974) and liver plasma membrane (Rubin *et al.*, 1973).

Microsomal membranes. Microsomal membrane contained lower total phospholipid per mg of protein (0.25–0.31 mg/mg of protein) and cholesterol (0.09 mg/mg of protein at day 0) than synapto-

Table 2. Effect of diet fat and duration of feeding on brain synaptosomal membrane content of phosphatidylethanolamine, ethanolamine plasmalogen, phosphatidylcholine, sphingomyelin and cholesterol

Values given are means $\pm$ S.D. '0 days' indicates rats were killed before diet treatment. For details of the diets, see the text. Analysis of variance and the effect of diet is indicated. Three replicates of four rats were killed for each treatment. Animals weighed 52.9 ± 3.7 g at 0 days and weighed the following after feeding SBO, SBO + choline, SBO lecithin, SFO, chow or LER respectively: 223 ± 19.5 ; 144 ± 20.4 ; 233 ± 26.2 ; 234 ± 19.9 ; 244 ± 10 ; 218 ± 20.3 g. Values without a common superscript are significantly different. Abbreviation: NS, not significant.

Diet treatment	Content (nmol/mg of protein)				
	Phosphatidyl-ethanolamine	Ethanolamine plasmalogen	Phosphatidylcholine	Sphingomyelin	Cholesterol
0 days	198 ± 14.6	168 ± 10.8	284 ± 26.5^{ac}	43.8 ± 2.9	480 ± 32.3^a
SBO	205 ± 17.4	185 ± 17.3	305 ± 13.2^{ac}	41.6 ± 7.3	509 ± 39.5^a
SBO + choline	208 ± 15.1	192 ± 20.0	252 ± 15.1^{cd}	39.4 ± 10.2	509 ± 59.7^a
SBO lecithin	193 ± 12.3	171 ± 24.8	330 ± 8.8^a	41.6 ± 5.8	672 ± 113^b
SFO	207 ± 6.2	204 ± 0.5	266 ± 9.5^{ce}	32.1 ± 5.1	414 ± 0.00^a
Chow	230 ± 14.6	187 ± 29.2	294 ± 19.5^{ade}	41.6 ± 13.9	526 ± 91.0^a
LER	211 ± 12.9	216 ± 29.2	249 ± 15.1^{bc}	48.2 ± 6.6	414 ± 25.8^a
Significant comparisons	NS	NS	$P < 0.01$	NS	$P < 0.05$

Table 3. *Effect of diet fat and duration of feeding on brain microsome content of phosphatidylethanolamine, lysophosphatidylethanolamine, phosphatidylcholine, sphingomyelin and cholesterol*

Values are means $\pm$ s.d. For details of diet treatment, see the legend to Table 2 and the text. Analysis of variance for effect of diet is indicated. Three replicates of four rats were killed for each treatment. Values without a common superscript are significantly different. Abbreviation: NS, not significant.

Diet treatment	Content (nmol/mg of protein)				
	Phosphatidyl-ethanolamine	Ethanolamine plasmalogen	Phosphatidyl-choline	Sphingomyelin	Cholesterol
0 days	86.2 $\pm$ 7.3	60.5 $\pm$ 1.6	140 $\pm$ 6.3 ^a	21.2 $\pm$ 3.7 ^a	224 $\pm$ 40.8 ^a
SBO	79.5 $\pm$ 8.4	57.2 $\pm$ 10.3	156 $\pm$ 33.4 ^{ac}	24.1 $\pm$ 2.9 ^{ab}	321 $\pm$ 113 ^{ab}
SBO + choline	82.3 $\pm$ 6.2	53.5 $\pm$ 1.6	193 $\pm$ 12.0 ^{bc}	27.0 $\pm$ 0.7 ^{ab}	396 $\pm$ 79.1 ^{ab}
SBO lecithin	83.4 $\pm$ 6.7	54.5 $\pm$ 4.3	230 $\pm$ 8.8 ^b	28.5 $\pm$ 2.2 ^b	508 $\pm$ 164 ^b
SFO	83.4 $\pm$ 2.8	55.1 $\pm$ 2.7	187 $\pm$ 17.0 ^{bc}	26.3 $\pm$ 0.7 ^{ab}	344 $\pm$ 98.0 ^{ab}
Chow	93.5 $\pm$ 8.4	51.8 $\pm$ 1.6	194 $\pm$ 7.6 ^{bc}	24.1 $\pm$ 2.9 ^{ab}	302 $\pm$ 59.7 ^a
LER	80.6 $\pm$ 2.8	54.5 $\pm$ 3.8	159 $\pm$ 23.9 ^{ab}	22.6 $\pm$ 0.7 ^a	293 $\pm$ 133 ^a
Significant comparisons	NS	NS	$P < 0.01$	$P < 0.05$	$P < 0.05$

somal membrane (Table 3). The molar cholesterol/phospholipid ratio (0.73 at day 0) is consistent with previous observations (Chapman, 1972; Lape-tina *et al.*, 1968). Relative levels of phospholipids were similar to those previously reported for rat brain microsomes (Dickerson, 1968) except that no cardiolipin or phosphatidic acid was detected in the present study.

Effect of diet on membrane lipid composition. Dietary treatment had no effect on the synapto-somal membrane content of phosphatidylethanol-amine, ethanolamine plasmalogen or sphingomyelin (Table 2). However, levels of phosphatidylcholine and cholesterol were altered by diet. Dietary modulation of synaptosomal phosphatidylcholine levels appeared to be mediated through the relative ω -6, ω -3 and monounsaturated fatty acid content of the diet fed. Feeding SBO lecithin (high in ω -6 and ω -3 fatty acids) resulted in a significant increase ($P < 0.01$) in synaptosomal phosphatidyl-choline compared with LER (high in C_{18:1(9)}), SBO plus choline and SFO (high in ω -6 fatty acids). The diet containing LER also resulted in lower mem-brane phosphatidylcholine content than in animals fed on chow or SBO diets. The addition of free choline to the SBO diet decreased synaptosomal phosphatidylcholine levels relative to SBO or SBO lecithin treatments and reduced body and brain weight gain after 24 days of diet treatment (see the legend to Table 2). Feeding diets containing SBO lecithin caused a significant increase in the synapto-somal cholesterol content (Table 2).

Microsomal phosphatidylcholine content in-creased over the duration of the experiment. Day 0 values were significantly different from results with SBO + choline, lecithin, SFO and chow diets. Simi-lar to synaptosomal membranes, microsomal phos-phatidylcholine levels increased on feeding SBO

lecithin compared with SBO and LER. The increase in phosphatidylcholine levels observed when SBO lecithin is fed compared with SBO implies that the choline head group may be involved in this effect on phospholipid composition. Alternatively the differ-ential effect of SBO and SBO lecithin could be due to higher levels of ω -6 fatty acids found in the latter (Table 1). The lecithin diet treatment also resulted in increased sphingomyelin content of microsomes when compared with levels at day 0 and after feeding LER. Feeding SBO lecithin increased micro-somal membrane cholesterol content versus results at day 0, or compared with chow or LER treat-ments. The overall effect of diet fat on synapto-somal and microsomal membrane lipid composition was similar, with minor variations likely due to differences in rates of synthesis and turnover of lipids within each membrane. In addition, a highly significant correlation was observed between mem-brane phosphatidylcholine and cholesterol levels in both synaptosomal and microsomal membranes (Fig. 1).

Several questions arise from these observations.

(i) Is dietary fat exerting an extrinsic irrepressible influence over phospholipid biosynthesis and turn-over or is the alteration in membrane lipid com-position resulting from some form of feedback control within the membrane biosynthetic pathway? (ii) Do changes in the fatty acids available for membrane phospholipid synthesis and thus changes in phospholipid fatty acid composition alter some specific physical characteristic of the membrane that results in a concomitant alteration in polar-head-group composition necessary to maintain mem-brane physical properties? If either of these mech-anisms apply, then at the molecular level it would appear from the present observations that dietary fatty acids affect phosphatidylcholine biosynthesis *in*

Fig. 1. Correlation between the cholesterol and phosphatidylcholine content of rat brain synaptosomes and microsomes. Regression lines were constructed for cholesterol content of both membranes versus phosphatidylcholine content. ** $P < 0.01$.

Scheme 1. Major metabolic pathways for the biosynthesis of phosphatidylcholine

Abbreviations: lyso-PtdEtn, lysophosphatidylethanolamine; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; lyso-PtdCho, lysophosphatidylcholine; PtdEtn, phosphatidylethanolamine.

in vivo. Biosynthesis *de novo* of phosphatidylcholine involves the conversions shown in Scheme 1. It is possible that altering dietary fat could affect the overall synthesis of phosphatidylcholine through changes in cellular pools of metabolites that activate or inhibit the controlling enzyme, cytidyltransferase (Vance & Choy, 1979). Dietary fat could also modulate phosphatidylcholine biosynthesis through the methylation pathway (Scheme 1). Bremer & Greenberg (1961) demonstrated very low levels of methyltransferase activity in rat brain microsomes.

However, more recently Crews *et al.* (1980) have demonstrated this methylation pathway in synaptosomal preparations of rat brain. Control of the transmethylation pathway appears to be mediated through levels of substrate or products of the reaction (Hoffman *et al.*, 1981a,b). It has also been suggested that methyltransferase shows a preference for unsaturated fatty acyl species (Le Kim *et al.*, 1973), with the major products of this pathway being highly unsaturated phosphatidylcholines (Trehwella & Collins, 1973; Salerno &

Beeler, 1973). Thus a mechanism is suggested whereby dietary fat could alter the fatty acid population of phosphatidylethanolamine, which in turn could determine the extent of methylation of phosphatidylethanolamine to phosphatidylcholine.

The effect of diet on membrane polar-head-group composition has received little attention. It is known that feeding diets containing high-eruric acid rapeseed oil causes an increase in cardiac mitochondrial phosphatidylcholine levels in comparison with diets containing SBO (Innis & Clandinin, 1981*b*) or peanut oil (Blomstrand & Svensson, 1974). Membrane phosphatidylcholine content is also known to alter after treatment with phenobarbital (Young *et al.*, 1971) or phenobarbitone (Davison & Wills, 1974). Alteration of growth temperature also affects the ratio of phosphatidylethanolamine to phosphatidylcholine in liver and muscle mitochondria of the carp (Wodtke, 1981; Van den Thillart & De Bruin, 1981). Matheson *et al.* (1980) have reported that in brain endothelial cell membranes phosphatidylethanolamine decreases with age and phosphatidylcholine increases. Thus the polar-head-group composition of membranes is not static and can be influenced by external factors. Whether these changes in lipid composition alter the physicochemical properties of the membrane or affect the activity of any membrane-mediated functions in brain is still unknown. The melting temperature of the fatty acyl chains of phosphatidylcholine is lower (implying higher mobility) than phosphatidylethanolamine when the fatty acids are saturated, but equivalent after incorporation of *cis*-unsaturated fatty acid (Van Dijck *et al.*, 1976). Therefore any change in the fluidity of the membrane caused by increasing phosphatidylcholine levels would depend to some extent on the fatty acyl composition of the newly synthesized phospholipid. Resolution of whether or not membrane physicochemical properties are affected would require physical studies on different membranes by n.m.r. or e.s.r. spectroscopy. In this regard, enzymic methylation of phosphatidylethanolamine in erythrocyte ghosts resulted in decreased membrane viscosity, suggesting that an alteration in the polar head group can effect membrane fluidity (Hirata & Axelrod, 1978). Phosphatidylcholine also has a different surface charge (zero at neutral pH) compared with phosphatidylethanolamine (Boggs, 1980). Therefore it is likely that increases in membrane phosphatidylcholine level (Tables 2 and 3) could affect the overall surface charge of the membrane and its interaction with peripheral and integral membrane proteins.

Cholesterol has two known effects on bilayer lipids. If present in sufficient quantities it will prevent the fatty acyl chains from undergoing a phase transition from the liquid crystalline to the gel state (Ladbrooke *et al.*, 1968). Cholesterol also reduces

chain mobility and the fluidity of membrane lipids (Seelig & Seelig, 1980). Cholesterol does not associate with all membrane lipids to an equal extent and shows a preference for associating with phosphatidylcholine within a mixture rather than phosphatidylethanolamine (Van Dijck *et al.*, 1976). In this regard, it is interesting to note the strong correlation between membrane phosphatidylcholine and cholesterol levels observed in the present study (Fig. 1). Cholesterol either does not affect or increases the chain melting temperature of phosphatidylcholine. However, it disrupts the intermolecular hydrogen-bonding of phosphatidylethanolamine and lowers its chain melting temperature. Cholesterol may also induce transition of phosphatidylethanolamine in natural mixtures from the lamellar to the hexagonal phase (Cullis *et al.*, 1978). Cholesterol is usually found in high quantities in membranes with low levels of phosphatidylethanolamine. Synaptosomal membranes are an exception to this general rule and it could be that the resultant destabilization of the membrane is a required feature for optimal function of such processes as exocytosis or membrane fusion that is known to be an integral function of these neural membranes (Boggs, 1980).

Phospholipid fatty acid composition of brain membranes

The major fatty acids of phosphatidylcholine from synaptosomal and microsomal membranes are C_{16:0}, C_{18:0} and C_{18:1(9)} (Table 4; and Breckenridge *et al.*, 1972; Cotman *et al.*, 1969; Sun, 1972). Plasmalogens were not separated from the remainder of the ethanolamine glycerophospholipids; thus peaks corresponding to the dimethyl acetal derivatives, which form on methylation of the acyl alk-1-enyl phospholipids, were also observed ('other' peaks, Table 4). These were not definitively identified, but these fatty acid derivatives are primarily of the C_{16:0}, C_{18:0} and C_{18:1} species (Cotman *et al.*, 1969). Synaptosomal and microsomal phosphatidylethanolamine contains more ω -6 and ω -3 polyunsaturated fatty acids than phosphatidylcholine. The levels of ω -6 and ω -3 fatty acids found in synaptosomal phosphatidylethanolamine [33–39% (w/w)] are somewhat lower than those previously reported (Cotman *et al.*, 1969; Breckenridge *et al.*, 1972). Levels of ω -6 and ω -3 fatty acids in synaptosomal phosphatidylethanolamine increase with age (Table 5) and thus this discrepancy is apparently due to differences in age of animals examined. The ω -6 and ω -3 fatty acid content of phosphatidylethanolamine was higher for microsomes [39–43% (w/w)] than synaptosomal membranes (Table 5; Sun, 1972).

Effect of diet and duration of feeding. The fatty acyl tail composition of synaptosomal phospho-

Table 4. Fatty acid composition of phosphatidylethanolamine and phosphatidylcholine isolated from brain synaptosomal and microsomal membranes

Animals were fed various dietary fats as described in the Materials and methods section. Values are grand means $\pm$ S.D. for all diet treatments. The significance level for an effect of diet treatment was determined by analysis-of-variance procedures. The significance level is indicated as follows: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

Fatty acid	Fatty acid content (% w/w)			
	Synaptosomal membrane		Microsomal membrane	
	Phosphatidylcholine	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylethanolamine
C _{16:0}	56.45 $\pm$ 2.72*	11.34 $\pm$ 3.56****	49.08 $\pm$ 1.94	8.72 $\pm$ 1.23
C _{16:1}	0.16 $\pm$ 0.13	0.50 $\pm$ 0.23****	0.31 $\pm$ 0.07	0.39 $\pm$ 0.20
C _{18:0}	11.39 $\pm$ 0.91	17.66 $\pm$ 2.62	10.84 $\pm$ 0.48*	19.83 $\pm$ 1.09
C _{18:1}	23.22 $\pm$ 1.62	9.36 $\pm$ 2.87*	25.11 $\pm$ 1.24**	10.74 $\pm$ 0.92
C _{18:2} (ω -6)	1.04 $\pm$ 0.42	0.64 $\pm$ 0.27	1.25 $\pm$ 0.31	0.60 $\pm$ 0.23
C _{18:3} (ω -6)	0.02 $\pm$ 0.04*	0.17 $\pm$ 0.12***	—	0.11 $\pm$ 0.05
C _{20:1} (ω -9)	0.28 $\pm$ 0.17	0.26 $\pm$ 0.16***	0.50 $\pm$ 0.10	0.33 $\pm$ 0.09
C _{20:3} (ω -6)	0.09 $\pm$ 0.09	0.25 $\pm$ 0.14**	0.27 $\pm$ 0.16	0.29 $\pm$ 0.13
C _{20:4} (ω -6)	4.19 $\pm$ 0.69	14.01 $\pm$ 0.99*	6.22 $\pm$ 0.81	13.5 $\pm$ 0.85
C _{22:4} (ω -6)	0.18 $\pm$ 1.60	3.69 $\pm$ 0.82***	0.66 $\pm$ 0.16	5.13 $\pm$ 0.44***
C _{22:5} (ω -6)	0.08 $\pm$ 0.16***	1.30 $\pm$ 0.99****	0.21 $\pm$ 0.29****	1.57 $\pm$ 1.21****
C _{22:5} (ω -3)	—	0.05 $\pm$ 0.08**	0.01 $\pm$ 0.04	0.12 $\pm$ 0.09**
C _{22:6} (ω -3)	1.46 $\pm$ 0.54*	15.59 $\pm$ 2.54**	3.12 $\pm$ 0.60	20.32 $\pm$ 1.78
Other	1.80 $\pm$ 0.61	16.3 $\pm$ 1.90	2.16 $\pm$ 0.5	18.40 $\pm$ 0.67

lipids was more responsive to dietary fat treatment than that of microsomes. The membrane fatty acid composition of phosphatidylethanolamine was altered more as a function of diet than that observed for phosphatidylcholine (Table 5). Analogous selectivity has been reported by other investigators (Koblin *et al.*, 1980; Matheson *et al.*, 1981). Synaptosomal membrane lipid presumably responds to a greater degree than that of microsomes because of an increased rate of membrane synthesis and turnover.

Synaptosomal membranes. Changes in fatty acid composition were observed independent of diet fed, which seemed to relate to age. Levels of C_{16:0}, C_{16:1} and C_{18:3} (ω -3) in the phosphatidylethanolamine fraction of synaptosomal membranes fell from 0 to 24 days post-weaning with a concomitant increase in the levels of C_{22:4} (ω -6) and C_{22:6} (ω -3). A similar rise in membrane content of C_{22:6} (ω -3) was observed with time in the phosphatidylcholine fraction of synaptosomal membranes. Membrane saturated-fatty acid content decreased as a function of age in the phosphatidylethanolamine and phosphatidylcholine fractions (Table 5). However, in the latter phospholipid, this decrease was significant only in animals fed on SBO plus choline, lecithin, SFO, chow and LER diets. The ω -6/ ω -3 ratio of both phosphatidylcholine and phosphatidylethanolamine decreased with age (Table 5) (Karlsson, 1975; Tahin *et al.*, 1981).

The fatty acyl composition of synaptosomal phosphatidylethanolamine was altered by diet. Dietary LER caused C_{18:3} (ω -3) to decrease compared with SBO, SBO plus choline and lecithin diets. Feeding SBO, chow or LER reduced the C_{20:1} (ω -9) content compared with results at day 0 and after other diet treatments. LER also decreased membrane C_{20:3} (ω -6) content as compared with results at day 0, and after SBO plus choline, lecithin and SFO diets. Levels of C_{20:4} (ω -6) increased over day 0 values after feeding SBO-plus-choline-, lecithin- and SFO-containing diets. Increases in C_{20:4} (ω -6) after feeding diets high in C_{18:2} (ω -6) have been reported previously (Tahin *et al.*, 1981). The LER treatment decreased C_{22:5} (ω -6) content in comparison with chow. Feeding SFO resulted in an increase in C_{22:5} (ω -6) compared with day 0 values and all other diet treatments. Total monounsaturated fatty acid content of phosphatidylethanolamine was reduced by feeding SBO plus choline or lecithin when compared with day 0 values and chow and LER diet treatments (Table 5). The lecithin diet resulted in the lowest content of monounsaturated fatty acids and LER diets resulted in the highest monounsaturated fatty acid content. The ω -6/ ω -3 ratio also increased in the phosphatidylethanolamine fraction of synaptosomal membranes of animals fed on SFO similar to that of the day 0 level (Table 5). Analogous effects of diets high in C_{18:2} (ω -6) and low in C_{18:3} (ω -3) have been reported in brain endothelial membrane phospho-

Table 5. *Effect of diet fat on the lipid composition of brain microsomes and synaptosomes*

Animals were fed various dietary fats as described in the Materials and methods section. Values are means $\pm$ s.d. Values without a common superscript are significantly different ($P < 0.05$). Abbreviations: Σ ats, sum of saturated fatty acids; Σ monos, sum of monounsaturated fatty acids; $\Sigma(\omega-6)$, sum of $\omega-6$ fatty acids; $\Sigma(\omega-3)$, sum of $\omega-3$ fatty acids; Σ monos, sum of monounsaturated fatty acids; $\Sigma(\omega-6)$, sum of $\omega-6$ fatty acids; $\Sigma(\omega-3)$, sum of $\omega-3$ fatty acids; U.I., unsaturation index.

Phosphatidylethanolamine (synaptosomal)		Day 0	SBO	SBO + choline	SBO lecithin	SFO	Chow	LER
Σ ats		49.2 $\pm$ 5.10 ^a	40.0 $\pm$ 1.41 ^b	35.2 $\pm$ 0.92 ^b	37.0 $\pm$ 6.38 ^b	35.4 $\pm$ 1.15 ^b	38.1 $\pm$ 2.38 ^b	41.6 $\pm$ 2.98 ^b
Σ monos		12.9 $\pm$ 1.82 ^c	11.1 $\pm$ 0.68 ^{bc}	6.67 $\pm$ 0.59 ^a	7.59 $\pm$ 1.91 ^{ab}	9.71 $\pm$ 2.31 ^{abc}	12.1 $\pm$ 1.83 ^c	11.8 $\pm$ 3.76 ^c
$\Sigma(\omega-6)$		16.5 $\pm$ 1.19 ^a	18.5 $\pm$ 1.05 ^{ab}	21.4 $\pm$ 0.54 ^c	21.0 $\pm$ 0.82 ^c	23.6 $\pm$ 1.34 ^d	19.4 $\pm$ 1.88 ^{bc}	18.5 $\pm$ 0.61 ^{ab}
$\Sigma(\omega-3)$		10.0 $\pm$ 1.51 ^a	14.5 $\pm$ 1.80 ^b	17.7 $\pm$ 0.34 ^b	17.4 $\pm$ 2.20 ^b	15.5 $\pm$ 0.70 ^b	16.4 $\pm$ 2.15 ^b	16.17 $\pm$ 1.83 ^b
$(\omega-6)/(\omega-3)$		1.65 $\pm$ 0.13 ^a	1.28 $\pm$ 0.12 ^b	1.20 $\pm$ 0.03 ^b	1.21 $\pm$ 0.16 ^b	1.52 $\pm$ 0.04 ^a	1.18 $\pm$ 0.06 ^b	1.14 $\pm$ 0.10 ^b
U.I.		138 $\pm$ 12.1 ^a	173 $\pm$ 15.3 ^b	197 $\pm$ 4.00 ^b	195 $\pm$ 16.3 ^b	200 $\pm$ 7.40 ^b	187 $\pm$ 18.8 ^b	181 $\pm$ 10.9 ^b
Phosphatidylcholine (synaptosomal)								
Σ ats		71.4 $\pm$ 0.77 ^c	69.8 $\pm$ 2.73 ^{bc}	66.6 $\pm$ 0.69 ^{ab}	67.8 $\pm$ 1.11 ^{ab}	66.0 $\pm$ 0.47 ^a	67.2 $\pm$ 2.25 ^{ab}	65.9 $\pm$ 2.40 ^a
Σ monos		23.2 $\pm$ 1.16	22.1 $\pm$ 3.89	23.1 $\pm$ 0.61	24.1 $\pm$ 0.56	24.0 $\pm$ 0.29	24.4 $\pm$ 0.33	24.3 $\pm$ 0.08
$\Sigma(\omega-6)$		3.76 $\pm$ 0.57 ^a	6.08 $\pm$ 2.01 ^{bc}	4.71 $\pm$ 0.32 ^{abc}	4.51 $\pm$ 0.74 ^{ab}	6.88 $\pm$ 0.25 ^c	5.36 $\pm$ 0.83 ^{abc}	5.85 $\pm$ 1.36 ^{bc}
$\Sigma(\omega-3)$		0.54 $\pm$ 0.09 ^a	1.73 $\pm$ 0.06 ^b	1.44 $\pm$ 0.01 ^b	1.46 $\pm$ 0.13 ^b	1.55 $\pm$ 0.27 ^b	1.57 $\pm$ 0.50 ^b	1.93 $\pm$ 0.82 ^b
$(\omega-6)/(\omega-3)$		7.03 $\pm$ 1.24 ^a	3.53 $\pm$ 1.00 ^b	3.27 $\pm$ 0.19 ^b	3.10 $\pm$ 0.59 ^b	4.53 $\pm$ 0.71 ^b	3.61 $\pm$ 0.90 ^b	3.30 $\pm$ 0.92 ^b
U.I.		40.8 $\pm$ 3.41 ^a	52.5 $\pm$ 1.10 ^{ab}	53.5 $\pm$ 0.05 ^{ab}	53.4 $\pm$ 2.99 ^{ab}	58.7 $\pm$ 2.86 ^b	53.2 $\pm$ 6.70 ^{ab}	56.9 $\pm$ 10.3 ^b
Phosphatidylethanolamine (microsomal)								
Σ ats		27.2 $\pm$ 1.62	30.1 $\pm$ 1.95	27.5 $\pm$ 0.72	28.2 $\pm$ 2.08	29.0 $\pm$ 0.17	28.9 $\pm$ 1.28	29.4 $\pm$ 2.25
Σ monos		11.6 $\pm$ 1.90	12.1 $\pm$ 0.68	11.0 $\pm$ 0.99	11.6 $\pm$ 0.30	10.5 $\pm$ 0.17	11.7 $\pm$ 0.52	11.9 $\pm$ 1.08
$\Sigma(\omega-6)$		21.5 $\pm$ 1.27 ^a	20.2 $\pm$ 1.19 ^{ab}	21.4 $\pm$ 0.41 ^a	21.1 $\pm$ 0.65 ^a	25.8 $\pm$ 2.22 ^c	20.4 $\pm$ 0.33 ^{ab}	18.6 $\pm$ 1.15 ^b
$\Sigma(\omega-3)$		21.3 $\pm$ 0.37	20.1 $\pm$ 1.41	22.1 $\pm$ 0.68	20.3 $\pm$ 0.94	18.3 $\pm$ 1.48	20.7 $\pm$ 2.11	20.6 $\pm$ 3.21
$(\omega-6)/(\omega-3)$		1.01 $\pm$ 0.05 ^{ab}	1.01 $\pm$ 0.03 ^{ab}	0.97 $\pm$ 0.01 ^{ab}	1.04 $\pm$ 0.04 ^b	1.41 $\pm$ 0.09 ^c	0.99 $\pm$ 0.09 ^{ab}	0.91 $\pm$ 0.08 ^a
U.I.		225 $\pm$ 7.70	213 $\pm$ 12.2	229 $\pm$ 5.4	216 $\pm$ 7.5	222 $\pm$ 11.4	217 $\pm$ 13.5	209 $\pm$ 23.0
Phosphatidylcholine (microsomal)								
Σ ats		57.9 $\pm$ 2.49	59.8 $\pm$ 0.69	59.0 $\pm$ 4.73	60.5 $\pm$ 0.52	61.5 $\pm$ 1.50	60.7 $\pm$ 1.53	60.4 $\pm$ 2.80
Σ monos		27.3 $\pm$ 1.70 ^c	27.3 $\pm$ 0.37 ^{bc}	25.0 $\pm$ 1.48 ^{ab}	25.2 $\pm$ 0.17 ^{ab}	24.0 $\pm$ 0.63 ^a	26.4 $\pm$ 0.45 ^{bc}	26.9 $\pm$ 0.47 ^{bc}
$\Sigma(\omega-6)$		8.90 $\pm$ 0.69	8.31 $\pm$ 0.22	9.19 $\pm$ 2.69	8.61 $\pm$ 0.57	9.49 $\pm$ 0.89	7.58 $\pm$ 1.08	7.81 $\pm$ 1.26
$\Sigma(\omega-3)$		2.96 $\pm$ 0.45	3.01 $\pm$ 0.00	3.26 $\pm$ 1.21	3.39 $\pm$ 0.46	2.73 $\pm$ 0.20	2.95 $\pm$ 0.72	3.33 $\pm$ 1.07
$(\omega-6)/(\omega-3)$		3.05 $\pm$ 0.38 ^{bc}	2.76 $\pm$ 0.07 ^{abc}	2.87 $\pm$ 0.23 ^{abc}	2.56 $\pm$ 0.34 ^{ab}	3.47 $\pm$ 0.25 ^c	2.62 $\pm$ 0.31 ^{ab}	2.45 $\pm$ 0.48 ^a
U.I.		80.9 $\pm$ 2.80	75.8 $\pm$ 0.82	78.3 $\pm$ 15.4	77.1 $\pm$ 4.73	76.1 $\pm$ 4.26	72.2 $\pm$ 7.65	75.8 $\pm$ 12.0

lipids of developing rats (Matheson *et al.*, 1981), total lipids of various subcellular fractions of rat liver, heart and brain (Tahin *et al.*, 1981) and mouse synaptosomal membranes (Koblin *et al.*, 1980). SBO plus choline diet increased levels of $C_{22:5}(\omega-3)$. Levels of $C_{16:0}$ in synaptosomal phosphatidylcholine decreased on feeding of SFO and LER, diets containing the lowest levels of saturated fatty acids. The $C_{18:3}(\omega-6)$ content increased with treatment of SBO plus choline.

Microsomal membranes. Dietary fat also affected the fatty acid tail composition of microsomal membranes. Feeding SFO increased $C_{22:4}(\omega-6)$ in phosphatidylethanolamine compared with that after all other treatments except SBO plus choline. LER treatment lowered the levels of this fatty acid compared with levels after SFO and SBO plus choline treatment. Changes in $C_{22:4}(\omega-6)$ are likely to be due to levels of $C_{18:2}(\omega-6)$ within the diet, SFO having the highest and LER the lowest. The $C_{22:5}(\omega-3)$ content of microsomal phosphatidylethanolamine increased by feeding lecithin compared with SBO, SBO plus choline and SFO. Feeding SFO also reduced levels of this fatty acid in comparison with LER and SBO plus choline. The effects of diet on $C_{22:5}(\omega-3)$ content are apparently related to the $C_{18:3}(\omega-3)$ content of the diet. SFO has the lowest $(\omega-3)$ -unsaturated fatty acid content, whereas lecithin and LER have the highest. As noted previously, SFO increased levels of $C_{22:5}(\omega-6)$ and LER treatments decreased membrane content of this fatty acid. The $\omega-6/\omega-3$ fatty acid ratio of microsomal phosphatidylethanolamine was raised by the SFO treatment and lowered by LER treatment compared with lecithin (Table 5). Feeding LER resulted in an increase in $C_{18:0}$ in microsomal phosphatidylcholine compared with at day 0. SFO also increased the $C_{22:5}(\omega-6)$ content of this phospholipid fraction. The $\omega-6/\omega-3$ fatty acid ratio of phosphatidylcholine was also increased by SFO compared with lecithin, chow and LER. This ratio was lowered significantly by LER treatments when compared with values at day 0.

Previous studies on the effect of diet fat on different membrane lipids have suggested that brain membranes are the most resistant to change (Tahin *et al.*, 1981). Experiments where essential fatty acid deficiency is induced and compared with diets high in $(\omega-6)$ - and $(\omega-3)$ -unsaturated fatty acids are normally conducted on animals for two generations (Koblin *et al.*, 1980) or for long periods of time (Sun & Sun, 1974). The present study shows significant changes in polar lipid content and phospholipid fatty acid composition after only 24 days of treatment. The developing rat brain may be more responsive to dietary influence than that of the adult (Matheson *et al.*, 1981). Maximal changes in phospholipid fatty

acid composition with diet are evident after only 3 weeks and tend to decrease with age (the present study; Tahin *et al.*, 1981).

The majority of diet effects observed can be related to the balance of fatty acids within the diets fed. The interplay of elongation and desaturation of available precursor fatty acids lead to changes in brain phospholipid fatty acyl composition (Table 5). Brain phospholipids are particularly susceptible to different levels of $\omega-6$ and $\omega-3$ fatty acids. It has been proposed that linolenic acid inhibits the formation of $C_{22:5}(\omega-6)$ from $C_{18:2}(\omega-6)$ and thus membrane levels of $C_{22:5}(\omega-6)$ increase in diets such as SFO, which contains low quantities of $C_{18:3}(\omega-3)$ (Matheson *et al.*, 1981). LER tends to decrease the $\omega-6/\omega-3$ fatty acid ratio as it has the highest levels of $C_{18:3}(\omega-3)$ and the lowest levels of $C_{18:2}(\omega-6)$ (Table 1). Strong correlations were observed between levels of various fatty acids altered by diet. As levels of $C_{16:0}$ increased, the content of $C_{20:4}(\omega-6)$ decreased in synaptosomal phosphatidylcholine and phosphatidylethanolamine for both membranes. Similarly, there was a consistent negative correlation between levels of $C_{18:1}$ and $C_{22:4}(\omega-6)$. A positive correlation was present between membrane levels of $C_{22:4}(\omega-6)$ and $C_{22:5}(\omega-6)$. These observations could be indicative of the interaction between fatty acid biosynthesis and precursor availability and the specific molecular substitution of one molecular species for another as a consequence of diet changes. The unsaturation index of both microsomal and synaptosomal membranes remained constant with all diet treatments tested but increased with age in synaptosomal phosphatidylethanolamine.

It is still not known whether these diet-induced changes in brain fatty acid composition and polar lipid content significantly alter the physical properties of these membranes and thus affect the molecular function of integral enzyme proteins. The interaction between diet, membrane polar-head-group content, cholesterol concentration, phospholipid fatty acid composition and the function of integral membrane proteins is likely to be extremely complex. However, in brain, as many enzymes of neurotransmitter metabolism are lipid dependent it is logical to postulate a possible interaction between dietary lipid and brain neurotransmitter metabolism. The cause-and-effect nature of changes in brain structural constituents remains to be resolved, but it is clear that the brain is sensitive to alteration of dietary lipid intake even in a nutritionally complete diet. Thus it is possible that degenerative disorders relating to alterations of brain structural lipid may potentially respond to dietary treatment via alteration of brain structural lipid and subsequent effects on the function of integral membrane proteins.

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