

Effect of dietary fatty acid composition on inositol-, choline- and ethanolamine-phospholipids of mammary tissue and erythrocytes in the rat

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The present study investigated the effect of feeding maize-oil, olive-oil and fish-oil diets, from weaning to adulthood, on rat mammary tissue and erythrocyte phospholipid fatty acid compositions. Effects of diet on the relative proportions of membrane phospholipids in the two tissues were also investigated. Mammary tissue phosphatidylinositol (PI) fatty acids were unaltered by diet, but differences in phosphatidylethanolamine (PE) and, to a lesser extent, phosphatidylcholine (PC) fractions were found between animals fed on different diets from weaning. Differences observed were those expected from the dietary fatty acids fed; *n*-6 fatty acids were found in greatest amounts in maize-oil-fed rats, *n*-9 in olive-oil-fed rats, and *n*-3 in fish-oil-fed rats. In erythrocytes the relative susceptibilities of the individual phospholipids to dietary modification were: PE > PC > PI, but enrichment with *n*-9 and *n*-3 fatty acids was not observed in olive-oil- and fish-oil-fed animals and in PC and PE significantly greater amounts of saturated fatty acids were found when animals fed on olive oil or fish oil were compared with maize-oil-fed animals. The polyunsaturated:saturated fatty acid ratios of PE and PC fractions were significantly lower in olive-oil- and fish-oil-fed animals. No differences in the relative proportions of phospholipid classes were found between the three dietary groups. It is suggested that differences in erythrocyte fatty acid composition may reflect dietary-induced changes in membrane cholesterol content and may form part of a homeostatic response the aim of which is to maintain normal erythrocyte membrane fluidity. The resistance of mammary tissue PI fatty acids to dietary modification suggests that alteration of PI fatty acids is unlikely to underlie effects of dietary fat on mammary tumour incidence rates.

Fatty acids: Mammary tissue: Phospholipids: Rat

Numerous studies using carcinogen-induced, spontaneous and transplantable mammary tumour models have shown that high-fat diets increase mammary tumour incidence and growth rates in animals (Welsch, 1985). These findings are frequently used to support human epidemiological evidence, some of which suggests increased incidence of breast cancer in populations and groups on high-fat diets (Armstrong & Doll, 1975; Carroll & Khor, 1975; Correa, 1981). Conflicting results reported in recent studies of the relationship between dietary fat and incidence of breast cancer demonstrate the uncertainty of this relationship (Byers, 1988; Berrino & Muti, 1989). Findings from some studies suggest that other related dietary characteristics, such as energy or essential fatty acid intakes, may be more important (Toniolo *et al.* 1989). For this reason there is a need to understand the mechanism by which changes in the content and composition of dietary fat leads to altered tumour incidence rates in animals. Such studies may indicate those dietary components which need to be investigated more closely in human populations.

Differences in mammary cell membrane phospholipid fatty acid composition in tumour-bearing animals on diets of varying fat content and fatty acid composition (Karmali *et al.* 1984; Jurkowski & Cave, 1985) and the high dependency of mammary tumour growth on

dietary linoleate (Ip, 1987) suggest that the cell membrane may act as a site for mediating effects of dietary fat on tumour incidence in animals. Dietary-induced alterations in membrane phospholipid fatty acid composition may be of particular significance in mammary tissue whose growth and development is known to be strongly influenced by steroid and peptide hormones (Welsch, 1987; Boyd & Leake, 1988).

Recognition of the importance of the minor membrane phospholipid phosphatidylinositol in mediating hormone signal transduction mechanisms involved in controlling cell proliferation and secretory processes (Nishizuka, 1984) led us to suggest that dietary-induced changes in phosphatidylinositol fatty acid composition may be of importance in determining effects of dietary fat on mammary tumour incidence in animals (Williams & Dickerson, 1987). The response of this membrane phospholipid to altered fatty acid intake has been studied in only a limited number of tissues and no studies have investigated effects of altered fatty acid intake on mammary membrane phosphoinositide fatty acid composition.

In the present study we have investigated the effect of dietary fatty acid intake on the composition of the two major membrane components, phosphatidylcholine and phosphatidylethanolamine, and on the minor component, phosphatidylinositol, in mammary tissue and erythrocytes of rats fed on diets of different fatty acid intake from weaning to adulthood. Because the content as well as the fatty acid composition of the phosphatidylinositol fraction may be important in determining cellular response to hormonal stimulation, we have also studied the effects of diet on the relative proportions of membrane phospholipids in the two tissues.

MATERIALS AND METHODS

Animals and diet

Twenty-four 30-d-old female Sprague Dawley rats weighing approximately 80 g were divided into three groups of eight animals to study effects of maize oil, olive oil and MaxEPA (fish oil) diets on membrane phospholipids. Animals were housed in groups of four in a room with controlled temperature, humidity and lighting. Animals were supplied with water and experimental diets *ad lib*. The basal diet contained (g/kg): starch 350, sucrose 300, casein 200, solka floc 40, mineral mix 40 (CaHPO₄, Na₂HPO₄, CaCO₃, MgSO₄, ZnCO₃, FeSO₄·7H₂O, CuSO₄·5H₂O, MnSO₄, H₂O), vitamin mix 20 (nicotinic acid, calcium-D-pantothenate, thiamin hydrochloride, riboflavin, pyridoxine, pteroylmonoglutamic acid, D-biotin, cyanocobalamin choline bitartrate) and methionine 2. Oils (maize oil, olive oil or MaxEPA) were added to the basal diet to provide 40 g/kg diet. The diets were analysed for their fatty acid compositions; the values are shown in Table 1. The diets were mixed weekly and stored at -20° until required. Food pots were changed daily to prevent adverse effects of peroxidation of fatty acids in the fish-oil diet. Daily food intakes and weekly body-weights were monitored for all the animals.

Animals were killed between 16 and 20 weeks of age with equal numbers of animals from each dietary group killed on the same day. Animals were anaesthetized using diethyl ether and blood (5–8 ml) removed by cardiac puncture and collected into tubes containing sodium citrate. The animals were killed by cervical dislocation, mammary tissue excised, weighed and taken to the laboratory for immediate extraction of total lipids using chloroform-methanol (2:1, v/v). Blood samples were centrifuged at 3000 g for 10 min and plasma and erythrocytes separated.

The packed erythrocytes were washed with saline (9 g sodium chloride/l) and lysed with distilled water. Propan-2-ol was added followed by chloroform to extract lipids. The extract

Table 1. *Fatty acid compositions (mol/100 mol) of maize-oil, olive-oil and fish-oil (MaxEPA) diets*

Diet ... Fatty acid	Maize oil	Olive oil	MaxEPA
16:0	10.3	5.2	16.5
16:1	0.1	2.6	11.8
18:0	1.4	2.1	3.8
18:1	24.7	72.2	12.9
18:2	60.7	12.2	3.8
18:3	1.2	0.9	3.1
20:3	0.2	0.5	6.5
20:4	0.1	0.0	0.7
20:5	0.6	0.4	19.4
22:4	0.1	0.7	1.3
22:5	0.0	0.2	1.5
22:6	0.2	0.3	10.2

was filtered to remove the cells, washed with 0.05 M-potassium chloride and the lower organic phase collected and dried under nitrogen. Separation of individual phospholipid classes in the lipid phases prepared from both tissues and blood cells was achieved by two-stage thin-layer chromatography as previously described (Williams & Maunder, 1989). Duplicate samples were run to allow analysis of both phospholipid content and fatty acid composition. The solvent system employed was found to achieve the best separation of serine and inositol phospholipids. Separated bands corresponding to sphingomyelin-, ethanolamine-, choline-, serine- and inositol-phospholipids were removed, the phospholipids hydrolysed and the phosphate determined (Bartlett, 1959) to allow quantification of the relative proportions of the phospholipid classes.

Bands corresponding to the ethanolamine-, choline- and inositol-phospholipids were removed from the other plated sample, the phospholipids hydrolysed, fatty acids methylated and subsequently analysed by gas-liquid chromatography (GLC). GLC analyses were carried out using a Varian 3400 instrument utilizing a 6 mm × 1.5 m glass column (10 g Silar 10C:100 g Gas Chrom Q (100–120 mesh)), initial oven temperature 165°, increased to 215° at 5°/min, injection temperature 250°, detector 275°, N₂ flow-rate 30 ml/min.

Results for individual fatty acids are expressed as mol/100 mol total fatty acids detected. The phospholipid polyunsaturated:saturated fatty acid (P:S) ratios were calculated from the fatty acid composition values and are presented with the tabulated values for phospholipid fatty acid composition. Results for phospholipid composition are expressed as mol/100 mol total phospholipid.

Statistical analysis of results

Values in the tables are presented as means and standard deviations, while values in figures are means with their standard errors. Analysis of variance was used to determine whether there were any differences between the three dietary groups with Duncan's range test used to determine the level of significance of any differences found. $P < 0.05$ was considered to be statistically significant. Where values were found to have an unacceptable level of variance, using Cochran's C, Bartlett Box or maximum/minimum variance tests, analysis was performed on log-transformed or square root-transformed data.

Table 2. Mean food intakes in animals fed on maize-oil, olive-oil and fish-oil (MaxEPA) diets†

(Values are means and standard deviations)

Diet ...	Food intake (g/animal per week)					
	Maize oil		Olive oil		MaxEPA	
	Mean	SD	Mean	SD	Mean	SD
	93.8	6.4	102*	6.8	96	8.5

* Mean value was significantly different from that for maize-oil and MaxEPA groups: $P < 0.05$.

† For details of diets, see p. 184 and Table 1.

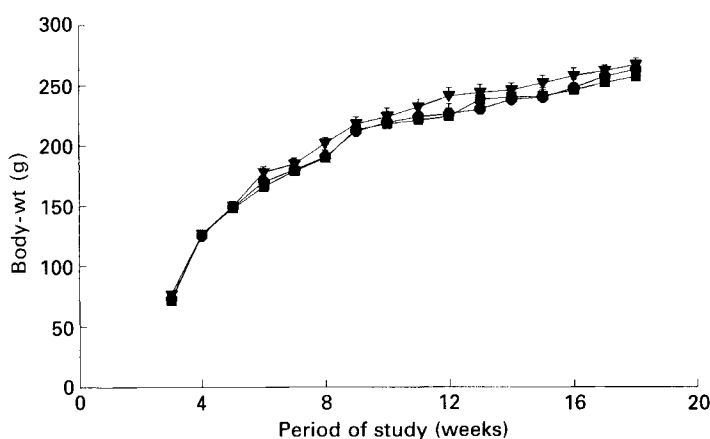


Fig. 1. Body-weights of rats in maize-oil- (▼), olive-oil- (■) and fish-oil (MaxEPA)- (●) fed groups from weaning to adulthood. For details of diets, see p. 184 and Table 1. Points are means with their standard errors, represented by vertical bars, for eight rats/group.

RESULTS

Mean food intakes over the period of study are shown in Table 2. Food intake was significantly higher in the animals fed on the olive-oil diet ($P < 0.05$), although growth rates and body-weights at death (Fig. 1) did not differ significantly between the three dietary groups.

Mammary tissue inositol-, choline- and ethanolamine-phospholipids

The fatty acid compositions of the inositol-, choline- and ethanolamine-phospholipid fractions from mammary tissue are shown in Table 3. There were no differences in the mammary phosphatidylinositol fatty acid composition between the three dietary groups. Only small differences in mammary phosphatidylcholine fatty acid composition were evident between the three groups, with significantly higher palmitoleic acid (16:1 $n-9$; $P < 0.05$) in the MaxEPA than maize-oil group, and significantly less docosatetraenoic acid (22:4 $n-6$; $P < 0.05$) in the MaxEPA group than the olive-oil group. The phosphatidylethanolamine fraction of mammary tissue showed the greatest response to dietary

Table 3. Fatty acid compositions of inositol-, choline- and ethanolamine-phospholipids from mammary tissue of rats fed on maize-oil, olive-oil and fish-oil (MaxEPA) diets

(Mean values and standard deviations for eight animals/group)

Fatty acid	Phosphatidylinositol						Phosphatidylcholine						Phosphatidylethanolamine					
	Maize oil		Olive oil		MaxEPA		Maize oil		Olive oil		MaxEPA		Maize oil		Olive oil		MaxEPA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0	22.9	5.5	26.9	2.2	27.1	2.1	46.4	10.6	51.9	5.6	49.7	11.1	18.0	2.9	21.4	5.8	21.7	5.1
16:1 n-9	2.1	1.9	2.1	1.6	1.2	1.6	0.4 ^a	0.5	0.7 ^{a,b}	0.7	1.7 ^b	0.9	3.3	1.1	3.8	1.5	3.2	1.6
18:0	31.1	8.2	28.3	5.7	28.5	4.8	19.3	3.8	19.3	3.8	19.6	2.0	19.4	3.8	20.0	4.2	19.7	3.9
18:1 n-9	13.8	9.8	10.5	3.7	7.7	6.9	6.1	3.6	8.1	3.5	11.0	5.7	14.2 ^a	4.6	18.3 ^b	2.2	14.8 ^a	1.9
18:2 n-6	3.4	3.0	1.9	0.7	4.9	6.9	5.2	3.7	2.1	1.4	3.7	2.0	5.9 ^a	3.0	2.6 ^b	0.7	2.3 ^b	0.7
18:3 n-3	2.3	1.0	3.0	1.1	3.4	1.8	1.4	1.9	1.1	0.8	0.7	0.3	1.5	2.2	1.7	0.9	1.5	0.7
20:3 n-6	5.5	6.9	5.3	1.8	4.7	1.7	4.1	4.4	2.4	1.5	1.2	0.4	6.9	1.7	7.6	1.2	8.7	2.1
20:4 n-6	7.5	3.8	7.3	1.9	9.1	3.5	6.8	4.2	4.3	1.6	2.9	1.8	13.3 ^a	4.3	10.3 ^{a,b}	5.2	7.8 ^b	2.8
20:5 n-3	1.2	1.4	1.6	0.9	1.6	1.1	0.7	0.7	0.7	0.9	2.1	2.3	4.0	1.9	2.9	1.9	5.9	4.6
22:4 n-6	0.9	1.0	1.2	0.5	1.1	0.9	0.5 ^a	0.6	1.1 ^b	1.2	0.1 ^a	0.1	4.4 ^a	2.4	3.0 ^{a,b}	2.8	1.4 ^b	1.5
22:5 n-3	2.4	1.2	2.8	0.7	3.7	2.2	3.7	2.9	2.0	0.8	2.1	0.4	1.8 ^a	1.1	1.8 ^a	0.8	3.9 ^b	2.0
22:6 n-3	3.0	1.6	4.5	1.8	4.2	1.2	2.3	0.9	2.3	1.1	2.7	2.2	2.2 ^a	0.7	2.6 ^a	0.9	4.4 ^b	1.6
P:S	0.5	0.1	0.5	0.1	0.6	0.1	0.5	0.4	0.2	0.1	0.2	0.1	1.1	0.3	0.9	0.4	0.9	0.5

P:S, polyunsaturated : saturated fatty acid ratio.

* For details of diets, see p. 184 and Table 1.

^{a, b} Values in same row within each phospholipid group with unlike superscripts were significantly different, *P* < 0.05.

Table 4. Fatty acid compositions of inositol-, choline- and ethanalamine-phospholipids in erythrocytes of rats fed on maize-oil, olive-oil and fish-oil (MaxEPA) diets*

(Mean values and standard deviations for eight animals/group)

Fatty acid	Phosphatidylinositol						Phosphatidylcholine						Phosphatidylethanolamine					
	Maize oil		Olive oil		MaxEPA		Maize oil		Olive oil		MaxEPA		Maize oil		Olive oil		MaxEPA	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
16:0	16.9 ^a	3.6	20.5 ^{ab}	4.7	22.7 ^b	4.9	31.6 ^a	9.1	46.9 ^b	9.9	58.7 ^c	5.6	18.2 ^a	1.6	27.9 ^b	6.6	28.8 ^b	5.5
16:1 n-9	1.2	0.5	1.9	2.0	1.3	1.3	0.9	0.8	0.5	0.7	0.7	0.2	3.8	0.9	2.9	1.1	3.6	1.3
18:0	35.7	3.7	36.1	7.5	44.8	13.3	16.8 ^a	8.9	27.8 ^b	7.2	26.3 ^b	3.6	9.6 ^a	0.6	14.7 ^b	2.8	16.1 ^b	2.6
18:1 n-9	8.5	2.1	9.7	3.7	6.3	2.5	7.1	4.5	9.5	3.0	5.8	4.4	14.7	2.9	15.7	4.6	11.8	4.2
18:2 n-6	2.6 ^a	0.4	1.3 ^b	0.5	1.1 ^b	0.2	2.5 ^a	0.8	2.3 ^a	1.1	1.1 ^b	0.8	3.7 ^a	0.5	2.2 ^b	0.8	1.5 ^b	0.2
18:3 n-3	1.4	1.0	2.0	1.5	1.4	0.9	3.2	5.3	0.4	0.3	0.2	0.1	1.8 ^a	0.4	2.6 ^a	1.4	4.5 ^b	1.6
20:3 n-6	2.2	1.5	3.3	1.1	2.7	1.4	4.6 ^a	2.7	1.9 ^b	2.9	0.5 ^b	0.3	6.3	3.2	6.8	2.5	7.9	1.7
20:4 n-6	22.3 ^a	9.3	13.2 ^b	7.8	7.5 ^b	4.9	13.6 ^a	7.2	3.5 ^b	1.7	1.4 ^b	0.8	20.4 ^a	1.8	11.9 ^b	3.6	5.3 ^c	2.1
20:5 n-3	1.1	1.4	1.5	1.2	2.8	3.3	2.3 ^a	2.4	0.6 ^a	1.0	1.0 ^b	1.2	3.8	2.8	3.9	2.1	6.1	4.2
22:4 n-6	1.0	0.4	1.3	1.1	0.6	0.5	1.1	2.1	0.4	0.2	0.4	0.5	8.2 ^a	1.9	2.4 ^b	3.6	0.5 ^b	0.6
22:5 n-3	1.0	0.7	1.7	1.1	1.9	1.4	7.1 ^a	3.5	1.5 ^b	1.7	1.0 ^b	0.6	1.4	0.5	1.6	2.3	2.5	2.4
22:6 n-3	1.9	0.7	3.4	2.2	3.5	1.9	2.4 ^a	0.8	1.1 ^b	0.8	1.0 ^b	0.4	1.3	0.6	2.3	0.9	2.4	1.4
P:S	0.7	0.2	0.5	0.2	0.4	0.2	1.0 ^a	0.4	0.2 ^b	0.2	0.1 ^b	0.04	1.7 ^a	0.2	0.8 ^b	0.3	0.7 ^b	0.3

P:S, polyunsaturated:saturated fatty acid ratio.

* For details of diets, see p. 184 and Table 1.

^{a, b, c} Values in same row within each phospholipid group with unlike superscripts were significantly different, $P < 0.05$.

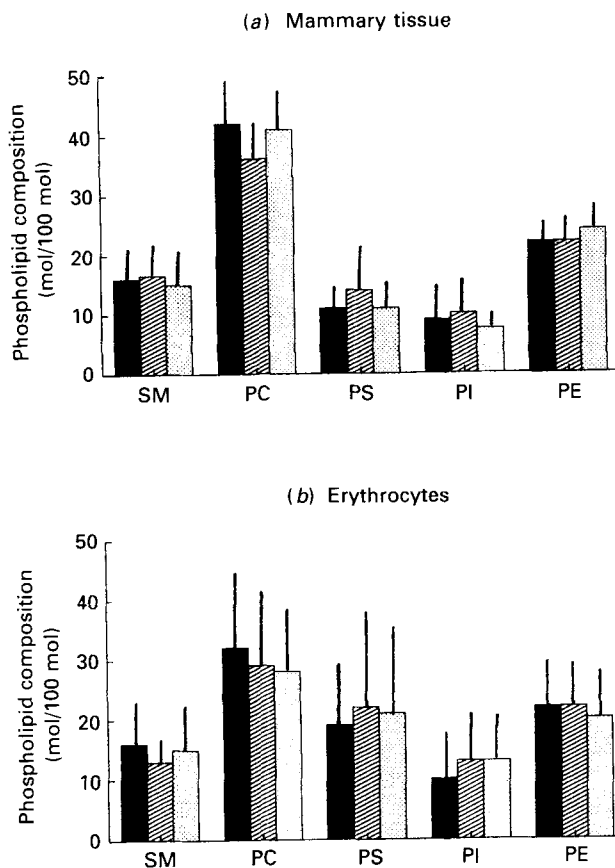


Fig. 2. Phospholipid compositions (mol/100 mol) of mammary tissue and erythrocytes of rats fed on maize-oil (■), olive-oil (▨) and fish-oil (MaxEPA) (▩) diets. For details of diets, see p. 184 and Table 1. Values are means with their standard errors, represented by vertical bars, for eight rats/group. SM, sphingomyelin; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine.

fatty acid intake, with higher relative proportions of oleic acid (18:1 *n*-9) in the olive-oil group than in the MaxEPA group ($P < 0.02$), and of linoleic acid (18:2 *n*-6) in the maize-oil than the olive-oil ($P < 0.02$) and the MaxEPA ($P < 0.02$) groups. Arachidonic (20:4 *n*-6) and docosatetraenoic (22:4 *n*-6) acids were also found in significantly larger amounts in animals fed on maize oil than in those fed on MaxEPA ($P < 0.1$ and $P < 0.05$ respectively). Mammary phosphatidylethanolamine of the MaxEPA group contained significantly more docosapentaenoic (22:5 *n*-3) and docosahexaenoic (22:6 *n*-3) acids than either the maize-oil or olive-oil groups ($P < 0.05$ in both cases).

Erythrocyte inositol-, choline- and ethanolamine-phospholipids

More marked differences in fatty acid compositions of all three phospholipids were found in the erythrocytes obtained from animals in the different dietary groups (Table 4). In the phosphatidylinositol fraction linoleic and arachidonic acids were present in significantly higher amounts in the maize-oil than the olive-oil or MaxEPA groups, with a particularly notable reduction in the arachidonate content of the MaxEPA group (7.50 (SD 4.90) v. 22.32

(SD 9.31); $P < 0.01$). Similarly, these fatty acids were found in decreasing quantities in the groups maize oil > olive oil > MaxEPA in the phosphatidylcholine and -ethanolamine fractions. Olive-oil feeding did not result in a significant incorporation of oleic acid into any of the phospholipids and *n*-3 fatty acids were not significantly increased in the phospholipid fractions of the erythrocytes of MaxEPA-fed animals. Indeed, in the phosphatidylcholine fraction there was significantly more of the fatty acids 22:5 *n*-3 and 22:6 *n*-3 in the maize-oil group than in either of the other two groups ($P < 0.01$ and $P < 0.02$). In the phosphatidylcholine and -ethanolamine of erythrocytes the most notable differences were in the relative proportions of saturated fatty acids palmitic acid (16:0) and stearic acid (18:0) which were higher in olive-oil- and MaxEPA-fed animals than in maize-oil-fed animals. These differences in saturated fatty acid content, together with the smaller amounts of arachidonic acid found in the MaxEPA and olive-oil groups, resulted in significant differences in the P:S ratios of the choline- and ethanolamine-phospholipids, the most notable differences being in the P:S ratios of the choline-phospholipid fractions of fish-oil-fed animals compared with maize-oil-fed animals ($P < 0.001$).

Phospholipid composition

Diet had no significant effect on the relative proportions of phospholipids in either mammary tissue or erythrocytes (Fig. 2). The phospholipid compositions of the two tissues were similar and no significant differences in phospholipid compositions were found between the two tissues in any of the dietary groups.

DISCUSSION

Mammary tissue phosphatidylinositol fatty acid compositions were remarkably similar in the three dietary groups. The P:S ratios of mammary phosphatidylinositol fatty acids were also very similar between the three dietary groups. In contrast, in mammary tissue ethanolamine- and choline-phospholipids there were differences in fatty acid composition which reflected the dietary fatty acids fed. The most notable differences between the three groups of animals were found in the ethanolamine-phospholipids. In this phospholipid *n*-6 fatty acids were found in the highest concentration in animals fed on maize oil from weaning, whereas oleic acid was found in greatest amounts in olive-oil-fed animals. The *n*-3 fatty acids, as might be expected, were found at their highest concentration in the fish-oil-fed animals. Similar trends for differences in fatty acid composition according to dietary group were evident in the phosphatidylcholine fraction, but most differences did not reach a level of statistical significance. One of the reasons for this may be that saturated fatty acids (16:0 and 18:0) comprise greater than 70 mol/100 mol total phosphatidylcholine fatty acids and, in this tissue, these fatty acids appear to be remarkably resistant to dietary modification. This means that the potential for changes in the relative proportions of other fatty acids is less than might otherwise be the case.

The observation in the present study that the fatty acid composition of mammary membrane phosphoinositide is resistant to dietary modification is in general agreement with results found for this membrane component in other tissues. In human platelets a number of investigators (Ahmed & Holub, 1984; Galloway *et al.* 1984) have demonstrated that in subjects supplemented with fish oils there is little incorporation of eicosapentaenoic acid (EPA) into platelet phosphatidylinositol, whilst EPA will readily displace arachidonic acid from choline- and ethanolamine-phospholipids in the same tissue. Gibney & Bolton-Smith (1988) similarly found that platelet inositol-phospholipids showed least changes in fatty acid composition in subjects taking dietary supplements of fish oils. However, some studies in animals have shown that it is possible to achieve dietary modification in the fatty

acid composition of this phospholipid. Weiner & Sprecher (1984) showed that in rat platelets and liver marked differences in phosphatidylinositol arachidonic acid composition could be achieved by feeding diets of different fatty acid composition, and earlier studies of Glenn & Dam (1965) had shown that the arachidonic acid content of chicken liver phosphatidylinositol was decreased by feeding linseed oil. These conflicting findings illustrate the diversity of response to changes in dietary fatty acid intake in different tissues and in different species.

It is, therefore, important that studies designed to investigate the relationship between dietary fat and breast disease measure fatty acid composition in this tissue rather than in those which are most easily accessible, such as erythrocytes and platelets in man. This conclusion is also supported by comparing the response to dietary fatty acid modification of erythrocytes and mammary tissue in animals in the present study. As in mammary tissue the phosphatidylinositol fraction showed the least, and phosphatidylethanolamine the greatest, response to dietary modification, with phosphatidylcholine intermediate between the two. The differences in erythrocyte phospholipids were, however, more marked than in mammary tissue, so that even in the phosphatidylinositol fraction significantly less *n*-6 fatty acids (18:2 *n*-6 and 20:4 *n*-6) were present in olive-oil- and fish-oil-fed animals compared with animals maintained on maize oil, and these differences were even more marked in the choline- and ethanolamine-phospholipid fractions. However, other differences in the fatty acid profiles of these two phospholipids were also observed, and these were less predictable from a knowledge of the fatty acid intakes of animals in the respective dietary groups. Thus, animals fed on an olive-oil diet showed no enrichment of oleic acid and animals fed on fish oils showed no enrichment of *n*-3 fatty acids in any of the erythrocyte phospholipids. There were, however, significantly greater amounts of both palmitic and stearic acids in choline- and ethanolamine-phospholipid fractions of animals fed on these two diets, and even in the inositol-phospholipid fraction the proportion of palmitic acid was significantly higher in animals fed on the fish-oil diet. The net effect of these changes is illustrated by the markedly lower P:S ratios in the choline- and ethanolamine-phospholipid fractions, most noticeable in the fish-oil group.

These responses are contrary to those which might be expected, since feeding a highly enriched polyunsaturated-fat diet such as a fish-oil diet might be expected to result in an increase in the membrane P:S ratio. The dramatic differences in phospholipid P:S ratios of animals in the present study, if unaccompanied by other differences in membrane composition, must result in differences in membrane fluidity. Thus, the higher P:S ratios of ethanolamine- and choline-phospholipids of olive-oil- and fish-oil-fed animals would be expected to cause decreased erythrocyte fluidity. It seems unlikely that diets rich in *n*-3 and *n*-9 fatty acids should cause decreased erythrocyte fluidity since this would result in decreased deformability and impaired ability of erythrocytes to pass through narrow vascular channels. Indeed, studies in human subjects suggest *n*-3 fatty acid supplements have beneficial effects on erythrocyte fluidity and have been shown to decrease erythrocyte viscosity in patients with peripheral arterial disease (Woodcock *et al.* 1984). Membrane fluidity is determined by the degree of unsaturation of phospholipid acyl chains, the phosphatidylcholine:sphingomyelin ratio and the cholesterol:phospholipid molar ratio (Borochoy *et al.* 1977; Smith, 1987). No differences in the phosphatidylcholine and sphingomyelin contents of membrane phospholipids were found between animals in the different dietary groups in the present study. Membrane cholesterol concentrations were not measured but feeding polyunsaturated fatty acids has previously been shown to increase liver membrane cholesterol content (Garg *et al.* 1985), and cholesterol feeding has been shown to alter erythrocyte fatty acid composition (Schouten *et al.* 1985). It has been suggested that these reciprocal changes may represent homeostatic responses to maintain

membrane fluidity, so that it is possible that the marked differences in the P:S ratios of erythrocyte phospholipids in the present study may be the result of dietary-induced differences in membrane cholesterol content. Further studies are required to determine whether this is the case and whether changes in membrane cholesterol content occur in tissues other than the erythrocyte.

Mammary and erythrocyte phospholipid compositions were not influenced by the type of fat fed and there were no significant differences in phospholipid composition between the two tissues. Other studies have shown that phospholipid composition is unaltered in response to changes in fat content and fatty acid composition (Hill *et al.* 1965; Ahmed & Holub, 1984). However, it was surprising that mammary tissue and erythrocyte phospholipid compositions were found to be similar since it is generally recognized that different tissues have specific phospholipid profiles which reflect the functions of different cells and organs (Wahle, 1983). Mean values for phosphatidylcholine were lower and those for phosphatidylserine were higher in erythrocytes than mammary tissue, but these differences did not reach statistical significance due to the high variances found for the phospholipid values in erythrocytes.

SUMMARY

Results of the present study support the view that the fatty acid composition of membrane phosphatidylinositol is relatively resistant to modification in response to dietary fatty acid. In particular the fatty acid composition of mammary tissue phosphatidylinositol seems to be highly conserved since no differences in fatty acid composition were found in tissues of animals fed on markedly different dietary fats from weaning to early adult life, although differences in the composition of other mammary membrane phospholipids were observed. These findings suggest that differences in phosphoinositide fatty acid composition are unlikely to underlie effects of dietary fat on mammary tumour incidence rates in experimental animals. Furthermore, they support the view that conservation of the fatty acid composition of this membrane phospholipid is related to its role as a second messenger in cell signal transduction mechanisms and that the maintenance of phosphatidylinositol fatty acid composition is an important feature of membrane homeostatic mechanisms. However, it is not known whether this homeostatic ability is lost in mammary tumour cells and further studies to consider this possibility are presently being undertaken. Mammary tissue choline- and ethanolamine-phospholipid fatty acids were found to be more responsive to dietary fat manipulation than the inositol-phospholipid fraction. These phospholipids are also involved in cell signal transduction mechanisms, so that dietary-induced alterations in these phospholipids cannot be excluded as a possible mechanism for effects of dietary fat on mammary tumourigenesis.

Differences in the way in which mammary tissue and erythrocyte phospholipids responded to dietary fatty acid intake were found in the present study. In mammary tissue, fatty acid profiles generally reflected dietary fatty acids with evidence of substitution of *n*-9, *n*-6, and *n*-3 fatty acids according to dietary enrichment. In erythrocytes, evidence of dietary substitution was much less, but in olive-oil- and fish-oil-fed animals there were increases in the saturated fatty acid contents of the major membrane phospholipids which may represent adaptive changes to maintain membrane fluidity. It is speculated that these responses may be secondary to effects of dietary fatty acids on membrane cholesterol concentrations. It is suggested that greater consideration should be given to the consequences of changes in membrane cholesterol content when considering effects of dietary fatty acids on cell function and on disease.

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