Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat

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A novel longitudinal feeding design was used to investigate the controlling influence of dietary fatty acids on the dynamic incorporation of fatty-acyl chains into phosphatidylcholine, phosphatidylethanolamine and cardiolipin in inner membrane of cardiac mitochondria. Rats were fed a polyunsaturated-fatty-acid-rich oil (soya-bean oil) for 12 days, crossed-over to a monounsaturated-fatty-acid-rich oil (rapeseed oil) for the next 11 days, then returned to soya-bean oil for 11 more days. Additional rats were fed either soya-bean oil or rapeseed oil only throughout. Rats were killed serially. Regression analysis was used to represent longitudinal flux in membrane lipid fatty-acid composition occurring with change in dietary fat. The fatty-acid composition of phosphatidylcholine, phosphatidylethanolamine and cardiolipin was influenced by dietary oil in a reversible way. Maximal diet influence was achieved in the 11-day cross-over period. Soya-bean oil to rapeseed oil cross-over caused the fatty-acid composition of phosphatidylcholine, phosphatidylethanolamine and cardiolipin to resemble that of rats fed rapeseed oil only. These changes were reversed by crossing back to soya-bean oil, indicating the dynamic state and short half-life of membrane phospholipid fatty-acyl chains. This report demonstrates for the first time in the whole animal fed diets adequate in all nutrients that subcellular membrane lipids rapidly respond to change in dietary fatty-acid balance. The system may be used to assess *in vivo* the significance of dietary fat in determining membrane physicochemical properties and biochemical functions.

Fatty-acid composition is a major factor influencing the physical state of membranes. A current model of the biomembrane is that of an asymmetric lipid bilayer with peripheral and integral membrane proteins arranged in transverse asymmetry across the membrane (Chapman *et al.*, 1979; Crain & Marinetti, 1979; Krebs *et al.*, 1979; Op den Kamp, 1979). Membrane-bound enzyme functions, lipid-requiring transport systems or protein–lipid interactions within the membrane may therefore be expected to be dependent on the physical properties of the phospholipids and at least partially on the nature of the fatty-acyl components of membrane phospholipids. The motional state of the lipid phase may physically determine the ability of the protein to undergo conformational changes necessary for optimal catalytic activity of the enzyme–substrate complex (Lenaz *et al.*, 1978; Sanderman, 1978). The Arrhenius activation energy of mitochondrial membrane-associated enzymes from homoeo-

thermic animals increases below the temperature limits of phase transition of the membrane lipid (Raison & McMurchie, 1974) further suggesting that cellular reactions involving membrane-associated enzymes are regulated by membrane fluidity. Alteration of membrane lipid fatty-acid composition results in a shift in transition temperatures (King *et al.*, 1977; McMurchie & Raison, 1979), implying that fluidity of the membrane can be modulated by altering the fatty-acyl chain components of membrane phospholipids.

In homoeothermic animals membrane lipid composition is influenced by the nature of dietary lipid. Diets deficient or very low in essential fatty acids produce well-defined changes in mitochondrial phospholipid with increases in $C_{20:3}$, $C_{18:1}$ and $C_{16:1}$ and decreases in $C_{18:2}$ and $C_{20:4}$ fatty-acyl chains, accompanied by a decrease in the activity of membrane-associated enzymes (Haeflner & Privett, 1975). On the other hand, diets rich in polyun-

saturated fatty acids increase the proportion of C_{18:2} fatty-acyl chains in mitochondrial phospholipid and decrease the Arrhenius activation energy of membrane-bound enzymes (McMurchie & Raison, 1979). Diets containing rapeseed oils with high amounts of long-chain monoenoic fatty acids result in marked changes in the fatty-acid composition of phospholipids from mitochondrial inner membrane and whole mitochondria of heart tissue (Blomstrand & Svensson, 1974; Clandinin, 1976; Renner *et al.*, 1979). This oil also produces decreased rates of ATP synthesis, ADP/O ratio and rate of energy transport by the membrane adenine nucleotide translocase/creatine phosphokinase (EC 2.7.3.2) energy transport system in isolated cardiac mitochondria (Clandinin, 1978, 1979; Renner *et al.*, 1979), together with pathological changes including necrosis and fibrosis of the heart muscle and changes in mitochondrial ultrastructure (Beare-Rogers, 1977; Yamashiro & Clandinin, 1980). Whether or not the latter events are the direct result of membrane fatty-acid composition is not known.

In view of the importance of dietary lipid as a mediator of lipid composition in mitochondrial inner membranes the present study was initiated to assess developmental changes in fatty-acyl chains of individual phospholipids that occur in the inner membrane of heart mitochondria when rats are fed diets rich in polyunsaturated fatty acids or rich in long-chain monoenoic fatty acids and to assess the possible reversible nature of these changes. A longitudinal cross-over design was used to demonstrate the dynamic nature of fatty-acyl chains in membrane lipids and their susceptibility to manipulation by dietary fat.

Materials and methods

Animals and diets

Male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Ont., Canada) weighing 55–60 g were fed diets containing 20% (w/w) soya-bean oil or rapeseed oil (Innis & Clandinin, 1980). The fatty-acid composition of the oils used is illustrated (Table 1). Rats were housed individually in a temperature-controlled room (21 ± 1°C). Diets and water were supplied *ad libitum*. Two replicates of rats were fed the diet containing soya-bean oil and two replicates were fed the diet containing rapeseed oil. Rats were killed by decapitation after 0, 2, 5, 8, 12, 17, 23, 28 and 34 days of dietary treatment. To ensure adequate sample size each group contained four rats before day 12 of the experiment, and thereafter three rats each. In addition, two replicates of rats were fed the diet containing soya-bean oil for 12 days, switched to the diet containing rapeseed oil for the next 11 days, then returned to the diet containing soya-bean oil for 11 more days. Of the

Table 1. *Fatty acid composition of oils*
Diets contained 20% (w/w) soya-bean oil (SBO) or 20% (w/w) rapeseed oil (RSO). Oils were of fully processed edible quality.

	Fatty-acid composition (% w/w)	
	SBO	RSO
C _{16:0}	10.0	4.5
C _{18:0}	2.2	1.4
C _{18:1}	23.6	23.6
C _{18:2}	55.7	16.6
C _{18:3}	8.5	7.6
C _{20:1}	0.0	13.3
C _{22:1}	0.0	33.3

Fig. 1. *Experimental design*

▲ indicates days on which rats were killed and the number of days on diet treatment; open bars indicate rats fed on a diet containing 20% (w/w) soya-bean oil; hatched bars indicate rats fed on a diet containing 20% (w/w) rapeseed oil.

latter, two groups of three rats were killed by decapitation on days 14, 17, 20 and 23 (rapeseed oil cross-over period) and on days 25, 28, 31 and 34 (soya-bean oil cross-over period) from the start of dietary treatment (Fig. 1).

Preparation of mitochondrial inner membrane

Rat hearts were quickly excised, cut into small pieces (approx. 2 mm³) and washed several times with an ice-cold solution of 0.21 M-mannitol containing 0.07 M-sucrose and 0.1 mM-EDTA. Hearts from the three or four rats for each group were then pooled and gently homogenized with a hand-type glass tissue grinder. The inner-membrane matrix compartment was prepared by differential centrifugation as described previously (Renner *et al.*, 1979) by the method of Schnaitman & Greenawalt (1968). Protein was measured by a colorimetric method (Lowry *et al.*, 1951).

Extraction and quantification of lipids

Inner-membrane matrix lipids were extracted in 20 ml of chloroform/methanol (2:1, v/v) containing

50 µg of ethoxyquin as antioxidant followed by 20 ml of chloroform/methanol/aq. NH₃ (35:5:2, by vol.) containing 50 µg of ethoxyquin, evaporated to dryness at 40°C under N₂, and dissolved in 0.2 ml of chloroform containing 2.8 mg of nonadecane/ml as an internal standard for quantification of lipid classes.

Total phospholipid, unesterified cholesterol, unesterified fatty acid, triacylglycerol and internal standard were quantified by using t.l.c. performed on thin silica-coated quartz rods (Chromarod S, Technical Marketing Associates, Mississauga, Ont., Canada), followed by flame-ionization detection in an Iatroscan TH-10 (Technical Marketing Associates). Lipid extract (2 µl) was applied to the base of each of four Chromarods per sample. Chromarods were developed for 30 min in light petroleum (b.p. 72.4–106.7°C)/diethyl ether (17:3, v/v) and air-dried for 20 min before detection of the separated lipids by passage of the rod through the flame of the detector. Differential and integral outputs were recorded by using 0.1 mV full-scale deflection with a chart speed of 0.254 m/min. The flame-ionization detector was operated with hydrogen flow rate of 80 ml/min, air flow of 2 litres/min at a scanning speed of 1 min per rod. Identification of lipid classes and calculation of response factors was achieved with known standards purchased from Supelco Inc. (Bellefonte, PA, U.S.A.).

Separation of phospholipids

Phospholipids were purified from the remaining total membrane lipid extract by separation from neutral lipids on silica gel G t.l.c. plates (20 cm × 20 cm) obtained from Analtech Inc. (Newark, DE, U.S.A.), with diethyl ether/benzene/ethanol/acetic acid (20:25:1:0.1, by vol.) and diethyl ether/hexane/acetic acid (25:75:1, by vol.) as sequential solvent systems. Individual phospholipids were separated in two dimensions on silica gel H t.l.c. plates (20 cm × 20 cm), obtained from the above supplier, with chloroform/methanol/28% aq. NH₃ (65:25:4, by vol.) followed by chloroform/acetone/methanol/acetic acid/water (30:40:10:10:1, by vol.) as solvent systems. Separated phospholipids were sprayed with 0.03% 2',7'-dichlorofluorescein in 0.01 M-NaOH and detected under u.v. light.

Fatty-acid analysis

Phosphatidylcholine, phosphatidylethanolamine and cardiolipin spots were scraped directly into test tubes with a known amount of heptadecanoic acid as internal standard. Phospholipid fatty acids were converted into their methyl esters by using boron trifluoride as described by Metcalfe & Schmitz (1961).

Fatty acid methyl esters were separated by g.l.c.

(Varian model 3700 gas chromatograph, Varian Canada, Georgetown, Ont., Canada) and quantified by using flame-ionization detectors. Chromatography was performed utilizing an open tubular glass capillary column (18 m × 0.25 mm internal diam.) coated with SP 1000 (grade AA column supplied by Supelco Inc.). Helium was used as the carrier gas at a column flow rate of 1.88 ml/min and inlet pressure of 80 kPa. The inlet splitter was set at 4 to 1. Samples were injected at 175°C and after 3 min the oven temperature was increased at a rate of 2°C/min for 20 min. Chromatography was completed within 50 min. Fatty acid methyl esters were identified with known standards and by the method of equivalent chain length (Miwa *et al.*, 1960). Identified fatty acids were comprised of the ω-3 series C_{18:3}, C_{20:5}, C_{22:5} and C_{22:6}; the ω-6 series C_{18:2}, C_{18:3}, C_{20:2}, C_{20:3}, C_{20:4}, C_{22:3}, C_{22:4} and C_{22:5}; the ω-7 series C_{16:1} and C_{16:2}; the ω-9 series C_{20:1}, C_{22:1} and C_{24:1} fatty acids and C_{14:0}, C_{16:0}, C_{18:0}, C_{20:0}, C_{22:0} and C_{24:0} saturated fatty acids. Values for C_{18:1} fatty acid represented total isomers. Peak areas, weight percentage fatty-acid composition and µg values based on the amount of internal standard were computed by a chromatography data system (CDS III, Varian Canada). To estimate change in the proportion of unsaturated bonds in the membrane, unsaturation index was calculated as:

$$\sum_{\alpha}^{\kappa} = (\text{number of double bonds in } \alpha) \times (\text{wt.\% occurrence of } \alpha \text{ for each fatty acid } \alpha \text{ in a group of } \kappa \text{ fatty acids}).$$

Mean chain length was calculated as:

$$\sum_{\alpha}^L (\text{number of carbon atoms in each fatty acid } \alpha) \times (\text{wt.\% occurrence of } \alpha \text{ for each fatty acid } \alpha \text{ in a phospholipid } L) / 100.$$

Data analysis

The influence of dietary fat on membrane phospholipid composition and its ability to rapidly effect alterations in membrane fatty-acyl chains was best suited to regression analysis. The clear description of flux in membrane lipid composition with time and with change in diet fat source given by this type of analysis is shown in examples (Fig. 2). A straight line was fitted to the data for each fatty acid against time on diet from days 12 to 34. The period up to day 12 was taken as a period of stabilization of membrane lipid composition to the dietary oil fed. On the basis of estimated fatty acid half-life in mitochondrial phospholipids (Landriscina *et al.*, 1976), more than 90% turnover of fatty-acyl chains might be expected in this 12-day period. Regression equations, reliability of the linear fit to the data and

Fig. 2. Dynamic changes in the fatty-acid composition of phosphatidylcholine, phosphatidylethanolamine and cardiolipin induced by diet

Regression lines were constructed for percentage content of individual fatty acids of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and cardiolipin (CL) isolated from mitochondrial inner membrane of rats fed soya-bean oil (SBO), rapeseed oil (RSO), crossed to rapeseed oil for days 12–23, after 12 days on soya-bean oil (RSO-X) or crossed to soya-bean oil for days 23–34, after 'RSO-X' regimen (SBO-X) versus time from days 12–34. Equations of these lines and standard deviations of data from each line are given in Table 4. Analysis for difference in slope of lines for soya-bean oil and rapeseed oil from each other and from zero, and of 'RSO-X' from 'SBO-X' regimen were performed as described in the Materials and methods section. In the above examples: (a) soya-bean oil versus zero, not significant; rapeseed oil versus zero, not significant; soya-bean oil versus rapeseed oil, not significant; 'RSO-X' versus 'SBO-X', $P < 0.001$; (b) soya-bean oil versus zero, $P < 0.001$; rapeseed oil versus zero, $P < 0.001$; soya-bean oil versus rapeseed oil, $P < 0.01$; 'RSO-X' versus 'SBO-X', $P < 0.01$; (c) soya-bean oil versus zero, not significant; rapeseed oil versus zero, $P < 0.001$; soya-bean oil versus rapeseed oil, not significant; 'RSO-X' versus 'SBO-X', $P < 0.001$; (d) soya-bean oil versus zero, not significant; rapeseed oil versus zero, $P < 0.001$; soya-bean oil versus rapeseed oil, $P < 0.001$; 'RSO-X' versus soya-bean oil, $P < 0.001$.

analysis of variance for the effect of diet treatment on the composition of each phospholipid were performed by computer using Minitab (Ryan *et al.*, 1976). A value of $P < 0.05$ was required to establish linearity. To compare treatment effects, difference in slope of the regression line was calculated for the following; rats fed soya-bean oil and rapeseed oil from days 12 to 34 versus each other and versus zero values, and for rats crossed from soya-bean oil to rapeseed oil from days 12 to 23 versus rats crossed back to soya-bean oil from rapeseed oil from days 23 to 34 (Steel & Torrie, 1960).

Results

In agreement with previous reports the fatty-acid composition of phosphatidylcholine, phosphatidyl-

ethanolamine and cardiolipin isolated from rat cardiac mitochondria (Blomstrand & Svensson, 1974; Dewailly *et al.*, 1977) or total rat heart tissue (Kramer *et al.*, 1979) were markedly different from one another. Results of the present study (Tables 2 and 3) show that phosphatidylcholine contained substantial amounts of ω -6 fatty acids ($C_{18:2}$ and $C_{20:4}$) and saturated fatty acids. Phosphatidylethanolamine was rich in long-chain derivatives of the ω -3 series, particularly $C_{22:6}$, contained substantially more $C_{20:4}$ than $C_{18:2}$ fatty acid and a high proportion of saturated fatty acids. Cardiolipin was characteristically high in ω -6 fatty acids, specifically $C_{18:2}$, and contained a much lower amount of saturated fatty acids than phosphatidylcholine and phosphatidylethanolamine (Tables 2 and 3).

Table 2. *Effect of diet oil on composition of lipids in inner membrane of rat heart mitochondria*

Values given are means $\pm$ S.E.M. for: 0 days, rats killed before dietary treatment; SBO, rats fed soya-bean oil 0–34 days; RSO, rats fed rapeseed oil 0–34 days; RSO-X, rats crossed to rapeseed oil for days 12–23 after days 0–12 on soya-bean oil; SBO-X, rats crossed from 'RSO-X' to soya-bean oil on days 23–34; $\Sigma_{\text{sats.}}$, sum of saturated fatty acids; $\Sigma_{\text{monos.}}$, sum of monounsaturated fatty acids; U.I., unsaturation index; $\bar{x}$ Ch.L., mean chain length. Values without a common superscript are significantly different ($P < 0.05$). Experimental details are given in the text.

	0 Days	SBO	RSO	RSO-X	SBO-X
Phosphatidylcholine					
$\Sigma_{\text{sats.}}$	53.0 $\pm$ 1.12 ^c	47.3 $\pm$ 0.92 ^{bc}	39.9 $\pm$ 1.74 ^a	41.6 $\pm$ 1.38 ^a	43.9 $\pm$ 1.58 ^{ab}
$\Sigma_{\text{monos.}}$	10.4 $\pm$ 0.40 ^a	8.1 $\pm$ 0.22 ^a	19.2 $\pm$ 0.78 ^c	16.3 $\pm$ 0.63 ^b	9.2 $\pm$ 0.67 ^a
$\Sigma(\omega-6)$	25.0 $\pm$ 3.26 ^{ab}	31.2 $\pm$ 0.90 ^{bc}	25.5 $\pm$ 2.02 ^a	24.2 $\pm$ 1.83 ^a	36.0 $\pm$ 1.53 ^c
$\Sigma(\omega-3)$	6.1 $\pm$ 0.52 ^c	4.8 $\pm$ 0.35 ^{bc}	3.4 $\pm$ 0.28 ^a	4.0 $\pm$ 0.47 ^{ab}	4.1 $\pm$ 0.48 ^{bc}
U.I.	137.1 $\pm$ 6.85	148.1 $\pm$ 4.00	137.2 $\pm$ 7.43	140.0 $\pm$ 3.85	157.6 $\pm$ 7.18
$\bar{x}$ Ch.L.	18.1 $\pm$ 0.09 ^a	18.5 $\pm$ 0.05 ^{ab}	18.8 $\pm$ 0.13 ^b	18.7 $\pm$ 0.07 ^b	18.4 $\pm$ 0.05 ^{ab}
Phosphatidylethanolamine					
$\Sigma_{\text{sats.}}$	57.3 $\pm$ 2.14 ^b	56.7 $\pm$ 0.81 ^b	47.9 $\pm$ 0.69 ^a	48.6 $\pm$ 1.63 ^a	54.1 $\pm$ 0.92 ^b
$\Sigma_{\text{monos.}}$	8.0 $\pm$ 0.51 ^a	6.5 $\pm$ 0.20 ^a	22.1 $\pm$ 0.94 ^c	16.6 $\pm$ 1.47 ^b	9.8 $\pm$ 0.98 ^a
$\Sigma(\omega-6)$	19.2 $\pm$ 1.53 ^a	26.6 $\pm$ 0.72 ^b	21.7 $\pm$ 1.55 ^a	23.0 $\pm$ 0.94 ^{ab}	28.5 $\pm$ 0.67 ^b
$\Sigma(\omega-3)$	9.0 $\pm$ 0.59 ^a	14.0 $\pm$ 0.75 ^{ab}	10.3 $\pm$ 1.36 ^{ab}	11.6 $\pm$ 0.87 ^{ab}	15.5 $\pm$ 1.21 ^b
U.I.	161.2 $\pm$ 12.20 ^a	205.9 $\pm$ 11.50 ^{ab}	179.7 $\pm$ 12.78 ^{ab}	198.7 $\pm$ 8.66 ^{ab}	219.0 $\pm$ 5.62 ^b
$\bar{x}$ Ch.L.	18.0 $\pm$ 0.17 ^a	19.0 $\pm$ 0.06 ^b	19.1 $\pm$ 0.10 ^b	19.2 $\pm$ 0.08 ^b	19.0 $\pm$ 0.04 ^b
Cardiolipin					
$\Sigma_{\text{sats.}}$	29.6 $\pm$ 2.17 ^c	16.3 $\pm$ 1.29 ^{ab}	17.0 $\pm$ 1.83 ^b	19.6 $\pm$ 1.68 ^b	9.3 $\pm$ 0.87 ^a
$\Sigma_{\text{monos.}}$	20.6 $\pm$ 2.08 ^c	9.5 $\pm$ 0.48 ^a	17.2 $\pm$ 0.91 ^c	15.8 $\pm$ 1.65 ^{bc}	12.3 $\pm$ 0.56 ^{ab}
$\Sigma(\omega-6)$	22.8 $\pm$ 0.78 ^a	44.3 $\pm$ 1.88 ^{cd}	38.7 $\pm$ 2.33 ^{bc}	29.9 $\pm$ 2.79 ^{ab}	52.9 $\pm$ 2.96 ^d
$\Sigma(\omega-3)$	5.6 $\pm$ 1.40 ^b	4.2 $\pm$ 0.20 ^{ab}	3.6 $\pm$ 0.15 ^b	3.8 $\pm$ 0.24 ^{ab}	3.8 $\pm$ 0.25 ^{ab}
U.I.	149.1 $\pm$ 11.0 ^a	182.1 $\pm$ 3.35 ^b	162.6 $\pm$ 4.78 ^a	158.1 $\pm$ 4.70 ^a	187.3 $\pm$ 2.64 ^b
$\bar{x}$ Ch.L.	18.6 $\pm$ 0.09 ^{ab}	18.8 $\pm$ 0.09 ^{ab}	19.2 $\pm$ 0.14 ^b	19.1 $\pm$ 0.18 ^{ab}	18.6 $\pm$ 0.04 ^a

Dietary and developmental effects on mitochondrial phospholipid fatty-acid composition

Mitochondrial membrane phospholipids isolated from animals killed before dietary treatment (day 0) contained a much higher proportion of C₁₄ and C₁₆ saturated fatty acids than rats killed later during the dietary treatments. The proportion of C_{14:0}, C_{16:0}, C_{18:0} at day 0 was as follows: in phosphatidylcholine, 1.72 $\pm$ 0.19, 28.2 $\pm$ 1.05, 22.7 $\pm$ 0.44 (% w/w) respectively; in phosphatidylethanolamine, 13.4 $\pm$ 1.42, 10.9 $\pm$ 0.76, 20.3 $\pm$ 1.01 (% w/w) respectively; in cardiolipin 0.85 $\pm$ 0.47, 14.1 $\pm$ 1.24, 11.4 $\pm$ 1.44 (% w/w) respectively. Values obtained for these fatty acids after dietary treatments are given in full (Table 3). Amounts of C_{14:0} and C_{16:0} fatty acids in all phospholipids, and amounts of C_{18:0} fatty acid in cardiolipin decreased with feeding of all treatment groups. However, total saturated fatty acid content decreased only in cardiolipin (Table 2).

Phosphatidylcholine fatty-acid composition. Dietary soya-bean oil had no effect on amounts of total saturated, monounsaturated, $\omega-6$ or $\omega-3$ fatty acids, unsaturation index (U.I.) or mean chain length of fatty acid present in phosphatidylcholine separated from mitochondrial inner membrane when compared with phosphatidylcholine from membranes of weanling rats killed at day 0 (Table 2).

Feeding 20% (w/w) rapeseed oil for 34 days, however, decreased saturated fatty acids from 53.0 to 39.9% and total $\omega-3$ fatty acids from 6.1 to 3.4%, whereas the monounsaturated fatty acid content was increased from 10.4 to 19.2% and the mean chain length was increased (Table 2). Comparison of rapeseed oil with soya-bean oil dietary treatments showed that mitochondrial phosphatidylcholine from rats fed rapeseed oil had a lower content of $\omega-3$, $\omega-6$ and saturated fatty acids and a higher content of monounsaturated fatty acids. Changing from soya-bean oil to rapeseed oil decreased $\omega-6$ and saturated fatty-acid content to amounts similar to those of rats fed rapeseed oil only. This cross-over elevated the monounsaturated fatty-acid content of phosphatidylcholine to midway between that observed for rats fed soya-bean oil and rapeseed oil only (Table 2). By changing back from rapeseed oil to soya-bean oil (day 23, Fig. 1), amounts of saturated, monounsaturated and $\omega-6$ fatty acids returned to values originally observed in soya-bean oil treatments.

Phosphatidylethanolamine fatty-acid composition. In contrast with phosphatidylcholine, the composition of mitochondrial phosphatidylethanolamine was altered by feeding soya-bean oil diets. Feeding this polyunsaturated-fatty-acid-rich oil increased the

Table 3. Control of fatty-acyl components in inner mitochondrial membrane phospholipids by diet

Values given are means $\pm$ S.E.M. for rats fed diets containing soya-bean oil (SBO) or rapeseed oil (RSO) from days 12 to 34 after metabolic adaptation to soya-bean oil or rapeseed oil from days 0 to 12. RSO-X indicates rats crossed to rapeseed oil from days 12 to 23 after days 0 to 12 on soya-bean oil. SBO-X indicates rats crossed to soya-bean oil from days 23 to 34 after 11 days under the 'RSO-X' regimen. Values without a common superscript are significantly different ($P < 0.05$). Experimental details are given in the text.

Fatty acid	Fatty-acid composition (% w/w)											
	Phosphatidylcholine						Phosphatidylethanolamine					
	SBO	RSO	RSO-X	SBO-X	RSO	RSO-X	SBO	RSO	RSO-X	SBO-X	RSO	RSO-X
C _{14:0}	0.5 $\pm$ 0.08	0.2 $\pm$ 0.05	0.6 $\pm$ 0.22	0.4 $\pm$ 0.06	0.9 $\pm$ 0.20	1.5 $\pm$ 0.39	0.1 $\pm$ 0.07	0.2 $\pm$ 0.06	0.1 $\pm$ 0.06	0.2 $\pm$ 0.06	0.2 $\pm$ 0.06	0.2 $\pm$ 0.06
C _{16:0}	13.0 $\pm$ 0.64 ^a	7.5 $\pm$ 0.66 ^a	8.8 $\pm$ 0.64 ^a	12.3 $\pm$ 0.53 ^b	4.7 $\pm$ 0.30 ^a	4.6 $\pm$ 0.55 ^a	4.7 $\pm$ 0.66 ^b	3.4 $\pm$ 0.42 ^{ab}	4.5 $\pm$ 0.37 ^{ab}	3.4 $\pm$ 0.42 ^{ab}	4.5 $\pm$ 0.37 ^{ab}	2.3 $\pm$ 0.21 ^a
C _{16:1} (ω -7)	0.1 $\pm$ 0.02 ^a	0.2 $\pm$ 0.04 ^{ab}	0.2 $\pm$ 0.04 ^{ab}	0.1 $\pm$ 0.03 ^{ab}	0.6 $\pm$ 0.24	0.8 $\pm$ 0.46	0.4 $\pm$ 0.04	0.2 $\pm$ 0.07	0.3 $\pm$ 0.09	0.2 $\pm$ 0.07	0.3 $\pm$ 0.09	0.3 $\pm$ 0.04
C _{16:2} (ω -7)	0.3 $\pm$ 0.05	0.3 $\pm$ 0.07	0.4 $\pm$ 0.06	0.3 $\pm$ 0.04	0.2 $\pm$ 0.02	0.2 $\pm$ 0.05	1.0 $\pm$ 0.10	0.8 $\pm$ 0.09	1.0 $\pm$ 0.10	0.8 $\pm$ 0.09	1.0 $\pm$ 0.10	0.6 $\pm$ 0.17
C _{18:0} (all isomers)	32.6 $\pm$ 1.08 ^b	26.3 $\pm$ 1.27 ^a	29.4 $\pm$ 1.19 ^{ab}	30.6 $\pm$ 1.20 ^{ab}	22.4 $\pm$ 0.64 ^a	24.6 $\pm$ 1.36 ^{ab}	27.4 $\pm$ 1.06 ^{bc}	5.6 $\pm$ 0.32 ^{ab}	8.1 $\pm$ 0.88 ^b	5.6 $\pm$ 0.32 ^{ab}	8.1 $\pm$ 0.88 ^b	4.2 $\pm$ 0.34 ^a
C _{18:1} (ω -6)	7.1 $\pm$ 0.18 ^a	10.4 $\pm$ 0.46 ^b	10.0 $\pm$ 0.37 ^b	7.5 $\pm$ 0.34 ^a	12.8 $\pm$ 0.34 ^d	10.0 $\pm$ 0.80 ^c	7.3 $\pm$ 0.52 ^b	7.7 $\pm$ 0.39 ^a	9.8 $\pm$ 0.60 ^b	7.8 $\pm$ 0.47 ^{ab}	9.8 $\pm$ 0.60 ^b	7.8 $\pm$ 0.42 ^{ab}
C _{18:2} (ω -6)	13.2 $\pm$ 0.34 ^b	10.2 $\pm$ 0.96 ^a	8.6 $\pm$ 0.65 ^a	14.6 $\pm$ 0.58 ^b	5.4 $\pm$ 0.36 ^a	4.8 $\pm$ 0.12 ^a	8.0 $\pm$ 0.40 ^b	40.4 $\pm$ 2.23 ^{bc}	25.5 $\pm$ 2.82 ^a	33.0 $\pm$ 3.16 ^{ab}	25.5 $\pm$ 2.82 ^a	48.1 $\pm$ 3.09 ^c
C _{18:3} (ω -6)	0.2 $\pm$ 0.04	0.4 $\pm$ 0.09	0.2 $\pm$ 0.03	0.1 $\pm$ 0.02	0.3 $\pm$ 0.03 ^b	0.2 $\pm$ 0.04 ^{ab}	0.3 $\pm$ 0.07	0.3 $\pm$ 0.04	0.4 $\pm$ 0.08	0.3 $\pm$ 0.04	0.4 $\pm$ 0.08	0.2 $\pm$ 0.03
C _{18:3} (ω -3)	0.3 $\pm$ 0.02 ^a	0.5 $\pm$ 0.04 ^b	0.4 $\pm$ 0.03 ^{ab}	0.3 $\pm$ 0.03 ^a	0.4 $\pm$ 0.03 ^c	0.4 $\pm$ 0.04 ^{bc}	0.6 $\pm$ 0.07	0.8 $\pm$ 0.11	0.8 $\pm$ 0.13	0.8 $\pm$ 0.11	0.8 $\pm$ 0.13	0.9 $\pm$ 0.06
C _{20:0} (ω -3)	0.2 $\pm$ 0.04 ^a	0.6 $\pm$ 0.10 ^b	0.6 $\pm$ 0.09 ^b	0.2 $\pm$ 0.02 ^a	0.4 $\pm$ 0.03 ^b	0.4 $\pm$ 0.06 ^b	0.2 $\pm$ 0.05	0.2 $\pm$ 0.03	0.4 $\pm$ 0.08	0.2 $\pm$ 0.03	0.4 $\pm$ 0.08	0.2 $\pm$ 0.05
C _{20:1} (ω -9)	0.1 $\pm$ 0.03 ^a	4.2 $\pm$ 0.13 ^d	3.3 $\pm$ 0.24 ^c	1.0 $\pm$ 0.22 ^b	4.4 $\pm$ 0.25 ^c	3.7 $\pm$ 0.56 ^c	1.4 $\pm$ 0.32 ^b	0.0 $\pm$ 0.01 ^a	2.8 $\pm$ 0.45 ^{bc}	3.0 $\pm$ 0.22 ^c	2.8 $\pm$ 0.45 ^{bc}	1.7 $\pm$ 0.19 ^b
C _{20:2} (ω -6)	0.3 $\pm$ 0.03 ^a	0.7 $\pm$ 0.09 ^b	0.4 $\pm$ 0.07 ^{ab}	0.2 $\pm$ 0.02 ^a	0.7 $\pm$ 0.17 ^b	0.4 $\pm$ 0.10 ^{ab}	0.3 $\pm$ 0.06 ^{ab}	0.7 $\pm$ 0.08	0.8 $\pm$ 0.10	0.7 $\pm$ 0.07	0.8 $\pm$ 0.10	0.9 $\pm$ 0.05
C _{20:3} (ω -6)	0.6 $\pm$ 0.08 ^{ab}	0.9 $\pm$ 0.14 ^b	0.6 $\pm$ 0.12 ^{ab}	0.3 $\pm$ 0.02 ^a	0.4 $\pm$ 0.05	0.4 $\pm$ 0.04	0.3 $\pm$ 0.03	1.0 $\pm$ 0.14 ^{ab}	1.1 $\pm$ 0.15 ^{ab}	1.5 $\pm$ 0.22 ^b	1.1 $\pm$ 0.15 ^{ab}	0.8 $\pm$ 0.08 ^a
C _{20:4} (ω -6)	15.8 $\pm$ 0.77	14.7 $\pm$ 1.78	13.8 $\pm$ 1.30	20.0 $\pm$ 1.60	16.6 $\pm$ 0.59	15.8 $\pm$ 0.80	17.6 $\pm$ 0.56	2.5 $\pm$ 0.26	2.0 $\pm$ 0.19	2.1 $\pm$ 0.30	2.0 $\pm$ 0.19	2.4 $\pm$ 0.18
C _{20:5} (ω -3)	0.7 $\pm$ 0.09	0.8 $\pm$ 0.14	0.7 $\pm$ 0.13	0.5 $\pm$ 0.05	0.4 $\pm$ 0.04 ^{ab}	0.6 $\pm$ 0.06 ^{bc}	0.3 $\pm$ 0.04 ^a	1.8 $\pm$ 0.15	2.0 $\pm$ 0.14	1.6 $\pm$ 0.12	2.0 $\pm$ 0.14	1.4 $\pm$ 0.20
C _{22:0}	0.0 $\pm$ 0.02	0.1 $\pm$ 0.07	0.0 $\pm$ 0.03	0.1 $\pm$ 0.03	0.1 $\pm$ 0.02	0.0 $\pm$ 0.02	0.1 $\pm$ 0.02	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.1 $\pm$ 0.11
C _{22:1} (ω -9)	0.0 $\pm$ 0.00 ^a	2.2 $\pm$ 0.12 ^b	2.2 $\pm$ 0.22 ^b	0.5 $\pm$ 0.11 ^a	2.8 $\pm$ 0.12 ^a	2.0 $\pm$ 0.28 ^c	0.8 $\pm$ 0.18 ^b	0.0 $\pm$ 0.00 ^a	4.3 $\pm$ 0.48 ^c	4.3 $\pm$ 0.48 ^c	1.9 $\pm$ 0.38 ^b	2.3 $\pm$ 0.20 ^b
C _{22:2} (ω -6)	0.4 $\pm$ 0.06	0.2 $\pm$ 0.11	0.2 $\pm$ 0.12	0.2 $\pm$ 0.03	0.2 $\pm$ 0.03	0.2 $\pm$ 0.04	0.2 $\pm$ 0.03	0.5 $\pm$ 0.07 ^c	0.0 $\pm$ 0.00 ^a	0.0 $\pm$ 0.00 ^a	0.0 $\pm$ 0.00 ^a	0.2 $\pm$ 0.08 ^b
C _{22:3} (ω -6)	0.5 $\pm$ 0.04 ^c	0.1 $\pm$ 0.03 ^a	0.2 $\pm$ 0.02 ^b	0.4 $\pm$ 0.05 ^c	0.4 $\pm$ 0.05 ^a	0.8 $\pm$ 0.10 ^b	1.2 $\pm$ 0.10 ^c	0.2 $\pm$ 0.05	0.1 $\pm$ 0.05	0.0 $\pm$ 0.03	0.1 $\pm$ 0.05	0.2 $\pm$ 0.06
C _{22:4} (ω -6)	0.1 $\pm$ 0.02 ^b	0.0 $\pm$ 0.00 ^a	0.1 $\pm$ 0.03 ^b	0.1 $\pm$ 0.01 ^b	0.6 $\pm$ 0.02 ^c	0.4 $\pm$ 0.05 ^b	0.6 $\pm$ 0.05 ^c	0.1 $\pm$ 0.05	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00	0.0 $\pm$ 0.00
C _{22:5} (ω -3)	1.3 $\pm$ 0.30 ^b	0.5 $\pm$ 0.05 ^a	0.7 $\pm$ 0.12 ^{ab}	1.0 $\pm$ 0.16 ^{ab}	2.4 $\pm$ 0.16 ^b	1.6 $\pm$ 0.09 ^a	2.3 $\pm$ 0.15 ^b	0.3 $\pm$ 0.02 ^b	0.1 $\pm$ 0.06 ^a	0.2 $\pm$ 0.05 ^a	0.1 $\pm$ 0.06 ^a	0.3 $\pm$ 0.04 ^{ab}
C _{22:6} (ω -3)	2.6 $\pm$ 0.26	1.7 $\pm$ 0.14	2.2 $\pm$ 0.26	2.4 $\pm$ 0.34	12.1 $\pm$ 0.42 ^{ab}	9.0 $\pm$ 0.77 ^b	12.6 $\pm$ 1.08 ^a	1.6 $\pm$ 0.13 ^b	0.8 $\pm$ 0.16 ^a	1.0 $\pm$ 0.17 ^a	0.8 $\pm$ 0.16 ^a	1.2 $\pm$ 0.08 ^{ab}
C _{24:0}	1.5 $\pm$ 0.34	3.8 $\pm$ 1.53	2.1 $\pm$ 0.62	2.4 $\pm$ 0.12	2.1 $\pm$ 0.54 ^b	11.7 $\pm$ 0.42 ^{ab}	0.2 $\pm$ 0.06 ^a	5.7 $\pm$ 1.10	6.6 $\pm$ 1.40	8.7 $\pm$ 2.32	6.6 $\pm$ 1.40	2.1 $\pm$ 0.73
C _{24:1} (ω -9)	0.6 $\pm$ 0.26	0.7 $\pm$ 0.29	0.7 $\pm$ 0.11	0.2 $\pm$ 0.06	0.1 $\pm$ 0.12	0.2 $\pm$ 0.12	0.1 $\pm$ 0.03	0.8 $\pm$ 0.26	1.0 $\pm$ 0.46	0.5 $\pm$ 0.21	1.0 $\pm$ 0.46	0.2 $\pm$ 0.07
C _{24:2}	0.7 $\pm$ 0.13	1.3 $\pm$ 0.35	1.2 $\pm$ 0.39	0.4 $\pm$ 0.06	0.9 $\pm$ 0.16	1.1 $\pm$ 0.24	0.4 $\pm$ 0.06	1.6 $\pm$ 0.39	2.3 $\pm$ 0.66	1.3 $\pm$ 0.16	2.3 $\pm$ 0.66	0.7 $\pm$ 0.22

proportion of total ω -6 fatty acids in phosphatidylethanolamine. Feeding the monounsaturated-fatty-acid-rich rapeseed oil had no effect on total percentage content of ω -6 series fatty acids, but decreased the content of saturated fatty acids and increased monounsaturated fatty acid content (Table 2). Thus, as observed for phosphatidylcholine, phosphatidylethanolamine isolated from mitochondrial inner membrane of rats fed rapeseed oil had lower saturated and ω -6 fatty-acid contents and higher monounsaturated fatty-acid content than phosphatidylethanolamine isolated from membranes of rats fed soya-bean oil. Alteration of dietary oil type from soya-bean oil to rapeseed oil resulted in a decrease in saturated fatty acids and an increase in unsaturated fatty acids. After crossing back from rapeseed oil to soya-bean oil these fatty acids returned to contents similar to those of rats fed soya-bean oil only (Table 2). Unlike phosphatidylcholine, the amount of total ω -6 fatty acids in phosphatidylethanolamine was not significantly altered by changing the nature of the oil consumed. Neither the type of dietary oil nor changing the oil had any significant influence on total ω -3 fatty acid content or U.I. of phosphatidylethanolamine. Mean fatty-acid chain length of phosphatidylethanolamine was increased over that of weanling rats killed at the start of the experiment to a similar extent by all treatments.

Cardiolipin fatty-acid composition. The relative proportion of saturated fatty acids fell and total ω -6 fatty acids rose in cardiolipin during the 34-day feeding period for both soya-bean oil and rapeseed oil treatments (Table 2). A marked increase in contents of $C_{18:2}$ fatty acid in cardiolipin occurred during the course of the experiment. $C_{18:2}$ represented $16.5 \pm 0.64\%$ of total fatty acids in cardiolipin isolated from cardiac mitochondria of weanling rats. By the end of the experiment (approx. 5 weeks post weaning) $C_{18:2}$ fatty acid approached a value of 50% total fatty acids in cardiolipin from rats fed either soya-bean oil or rapeseed oil only. Clearly the age of the animal examined influences the $C_{18:2}$ content observed for this phospholipid. When fed soya-bean oil for 34 days monounsaturated-fatty-acid content decreased and U.I. increased in mitochondrial cardiolipin. Rats fed rapeseed oil for 34 days had similar monounsaturated-fatty-acid content and U.I. when compared with weanling rats. Substitution of rapeseed oil for dietary soya-bean oil caused a decrease in ω -6 fatty acids and U.I. and an increase in monounsaturated fatty acids in mitochondrial cardiolipin. These changes were reversed by returning the rats to the soya-bean oil diet (Table 2). Neither the content of total ω -3 fatty acids nor mean chain length in cardiolipin was influenced by feeding soya-bean oil or rapeseed oil for 34 days, or

by substituting these oils for one another in the diet (Table 2).

The effect of dietary oil, and of changing the dietary oil, on fatty-acid composition of the individual phospholipids from heart mitochondrial inner membrane indicated no effect on mean values of $C_{14:0}$, $C_{16:2}$, $C_{20:4}$, $C_{22:0}$ or $C_{24:1}$ fatty acids in any phospholipid examined (Table 3). Overall changes in fatty-acid composition between membrane phosphatidylcholine and phosphatidylethanolamine induced by each dietary lipid source tended to be similar. For both of these phospholipids, continued feeding of rapeseed oil resulted in decreased amounts of $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, $C_{22:4}$, $C_{22:5}$ (ω -6 series) and $C_{22:5}$ (ω -3 series), whereas amounts of $C_{18:1}$, $C_{18:3}$ (ω -3 series), $C_{20:0}$, $C_{20:1}$ and $C_{20:2}$ fatty acids were increased relative to rats fed soya-bean oil. Elevation of $C_{18:3}$ (ω -3) and $C_{20:2}$ (ω -6) fatty acids in phosphatidylcholine and phosphatidylethanolamine by rapeseed oil is noteworthy in view of decreased amounts of the longer-chain derivatives of the (ω -3)- and (ω -6)-series fatty acids. Rapeseed oil increased the proportion of $C_{18:3}$ (ω -6) and $C_{20:5}$ fatty acids in phosphatidylethanolamine, but had no such effect on phosphatidylcholine. Amounts of $C_{16:1}$, $C_{20:3}$, $C_{22:3}$, $C_{22:6}$ and $C_{24:0}$ fatty acids in phosphatidylcholine and phosphatidylethanolamine were similar between rats fed soya-bean oil and rapeseed oil. Modulation of cardiolipin fatty-acid composition by the fat ingested was different from that observed for phosphatidylcholine and phosphatidylethanolamine. Rapeseed oil, relative to soya-bean oil, did not suppress the amount of the saturated fatty acids $C_{16:0}$ and $C_{18:0}$, nor did it increase $C_{18:3}$ (ω -3), $C_{20:0}$ or $C_{20:2}$ content in cardiolipin as in both phosphatidylcholine and phosphatidylethanolamine (Table 3). In cardiolipin, $C_{20:1}$ and $C_{22:1}$ fatty-acid content was increased and $C_{22:3}$, $C_{22:5}$ (ω -3) and $C_{22:3}$ (ω -6) contents were decreased by rapeseed oil in comparison with soya-bean oil. Amounts of all other fatty acids in cardiac mitochondrial cardiolipin were similar between rats fed soya-bean oil and rapeseed oil for 34 days.

Dynamic turnover of phospholipid fatty-acyl chains

The use of a cross-over design (Fig. 1) in this experiment provided a model enabling clear demonstration that the major fatty acids of phosphatidylcholine, phosphatidylethanolamine and cardiolipin are readily and reversibly modulated by dietary fat (Fig. 2). By use of regression analyses for each treatment period the rate of change of time frame for fatty-acid replacement in each phospholipid can be approximated (Fig. 2, Table 4). By calculation from graphical presentation of the data it is apparent that the half-life of the major fatty acids in phosphatidylcholine, phosphatidylethanolamine and

Values are given for linear-regression equation ($=y$) between fatty-acid content (% total fatty acids) and time in days on diet (x). The superscript value indicates $\pm$ s.d. Abbreviations used: SBO, rats fed a diet containing soya-bean oil from days 12 to 34; RSO, rats fed a diet containing rapeseed oil from days 12 to 34; RSO-X, rats crossed to rapeseed oil diet on days 12 to 23; SBO-X, rats crossed to soya-bean oil diet on days 23 and to 34 after 11 days on the 'RSO-X' regimen; ns, no significant effect of diet on fatty acid (% total fatty acids); nL, linear regression equation could not be fitted to the data ($P < 0.05$). Experimental details are given in the text.

Fatty acid	Phosphatidylcholine			Phosphatidylethanolamine			Cardiolipin			
	SBO	RSO-X	SBO-X	SBO	RSO	RSO-X	SBO	RSO	RSO-X	SBO-X
16:0	10.1	4.2	4.20	5.42	nL	9.9	6.72	5.27	7.95	7.62
16:1 (ω-7)	-0.014	0.018	0.49	+0.042 ^{x1.00}	ns	-0.28 ^{x1.23}	-0.083 ^{x2.00}	-0.084 ^{x1.20}	-0.16 ^{x1.75}	-0.17 ^{x0.69}
18:0	29.5	20.9	13.70	ns	ns	33.00	10.50	6.69	12.10	6.01
18:1 (all isomers)	+0.14 ^{x0.41}	+0.24 ^{x1.74}	-0.21 ^{x2.03}	nL	nL	-0.42 ^{x1.17}	-0.14 ^{x1.17}	-0.058 ^{x0.97}	-0.23 ^{x2.29}	-0.058 ^{x0.86}
	6.68	7.94	18.30	nL	11.70	-0.25	nL	6.14	6.36	nL
18:2 (ω-6)	+0.017 ^{x0.59}	+0.11 ^{x1.24}	-0.36 ^{x0.98}	+0.044 ^{x1.07}	+0.55 ^{x1.64}	+0.59	4.80	7.82	39.40	-26.9
18:3 (ω-6)	+0.049 ^{x1.08}	+0.29 ^{x2.04}	nL	2.72	4.54	5.59	24.0	7.82	39.40	+2.48 ^{x2.48}
18:3 (ω-3)	ns	ns	ns	+0.12 ^{x0.64}	+0.11 ^{x0.87}	-0.035 ^{x0.46}	+0.68 ^{x0.45}	+1.11 ^{x4.46}	-0.75 ^{x1.51}	ns
	0.23	nL	1.11	0.15	0.28	0.08	ns	ns	ns	ns
18:3 (ω-3)	+0.0044 ^{x0.08}	+0.045 ^{x0.08}	-0.026 ^{x0.06}	-0.0018 ^{x0.85}	+0.0074 ^{x0.06}	+0.0075 ^{x0.99}	+0.0061 ^{x0.11}	ns	ns	ns
20:0	nL	1.06	1.29	nL	0.53	0.08	ns	ns	ns	ns
20:1 (ω-9)	nL	-0.020 ^{x0.30}	-0.034 ^{x0.11}	0.07	5.93	-3.92	-0.039	nL	-2.19	4.39
20:1 (ω-9)	4.76	-0.209	8.20	+0.0018 ^{x0.06}	-0.0063 ^{x0.07}	+0.018 ^{x0.14}	+0.0086 ^{x0.11}	+0.021 ^{x0.031}	+0.26 ^{x1.17}	-0.086 ^{x0.54}
20:2 (ω-6)	0.46	-0.024 ^{x0.38}	-0.24 ^{x0.64}	nL	0.014	-0.09	ns	ns	ns	ns
20:2 (ω-6)	-0.0099 ^{x0.29}	+0.028 ^{x0.17}	-0.22 ^{x0.12}	+0.0018 ^{x0.05}	+0.028 ^{x0.28}	ns	2.20	2.90	2.15	1.36
20:3 (ω-6)	1.13	2.01	0.73	ns	ns	ns	-0.048 ^{x0.20}	-0.060 ^{x0.54}	-0.53 ^{x0.45}	ns
20:3 (ω-3)	-0.024 ^{x0.17}	-0.047 ^{x0.21}	-0.012 ^{x0.16}	nL	0.87	0.19	ns	ns	ns	ns
20:5 (ω-3)	ns	ns	ns	ns	-0.0092 ^{x0.21}	+0.19 ^{x0.16}	ns	ns	ns	ns
20:5 (ω-3)	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
22:0	0.00	2.80	4.53	0.00	3.34	-2.28	0.00	1.46	-2.98	3.56
22:1 (ω-9)	+0.00 ^{x0.00}	+0.15 ^{x0.91}	-0.13 ^{x0.43}	+0.00 ^{x0.00}	-0.023 ^{x0.22}	-0.22 ^{x0.39}	+0.00 ^{x0.00}	+0.12 ^{x1.18}	+0.26 ^{x0.60}	-0.043 ^{x0.45}
22:1 (ω-6)	ns	ns	-0.25	ns	ns	ns	0.73	0.00	0.92	-1.11
22:1 (ω-6)	nL	nL	0.74	ns	ns	ns	-0.0078 ^{x0.22}	+0.00 ^{x0.00}	-0.045 ^{x0.30}	+0.045 ^{x0.10}
22:2 (ω-6)	ns	ns	-0.025 ^{x0.12}	1.75	0.68	2.81	ns	ns	ns	ns
22:3 (ω-6)	0.45	0.45	-0.07	0.75	-0.011 ^{x0.12}	-0.11 ^{x0.20}	-0.78	ns	ns	ns
22:3 (ω-6)	-0.019 ^{x0.07}	-0.019 ^{x0.07}	+0.0053 ^{x0.04}	0.75	0.34	1.17	ns	ns	ns	ns
22:3 (ω-3)	2.09	+0.00 ^{x0.00}	-1.99	1.65	-0.0065 ^{x0.09}	-0.039 ^{x0.09}	-0.049	ns	ns	ns
22:3 (ω-3)	+0.011 ^{x0.14}	-0.075 ^{x0.21}	+0.101 ^{x0.21}	1.65	0.77	2.75	-0.32	nL	0.81	-0.60
22:4 (ω-3)	ns	ns	ns	+0.032 ^{x0.44}	+0.042 ^{x0.37}	-0.059 ^{x0.24}	+0.086 ^{x0.43}	nL	-0.037 ^{x0.06}	+0.028 ^{x0.10}
22:4 (ω-3)	0.13	ns	ns	9.64	2.09	13.00	-0.64	nL	3.07	-0.038
22:5 (ω-3)	+0.050 ^{x0.91}	+0.34 ^{x1.43}	+0.11 ^{x0.92}	+0.11 ^{x0.92}	+0.34 ^{x1.43}	-0.21 ^{x2.10}	+0.45 ^{x2.54}	nL	-0.12 ^{x0.36}	+0.039 ^{x0.13}
22:6 (ω-3)	ns	ns	ns	5.33	6.01	5.64	1.55	ns	ns	ns
22:6 (ω-3)	ns	ns	ns	-0.15 ^{x0.92}	-0.17 ^{x1.02}	-0.20 ^{x1.18}	-0.04 ^{x0.22}	ns	ns	ns

cardiolipin is 2–3 days, thus enabling complete or nearly complete replacement of major fatty-acyl chains within the treatment time frames employed. Replacement–repletion plots indicated that the half-life of fatty-acyl groups in cardiolipin was somewhat longer than observed for phosphatidylcholine and phosphatidylethanolamine (approx. 3 days versus 2.5 days respectively), and similarly that the observed half-life of non-essential fatty acids was somewhat longer than for the essential fatty acids of membrane phospholipids.

Changes in mitochondrial membrane phospholipid, total saturated, monounsaturated, ω -6 and ω -3 fatty acids, U.I. and mean chain length by altering the dietary lipid (Table 2) have been discussed. Specifically, changing from soya-bean oil to rapeseed oil (day 12, Fig. 1) resulted in a decrease in the contents of $C_{16:0}$, $C_{18:2}$ and $C_{22:4}$ and an increase in $C_{18:1}$, $C_{20:0}$, $C_{20:1}$ and $C_{22:1}$ fatty acids in both phosphatidylcholine and phosphatidylethanolamine (Table 3). A relative increase of $C_{16:1}$ in phosphatidylcholine and $C_{18:3}$ (ω -3) in phosphatidylethanolamine and a decrease of $C_{22:5}$ (ω -3) and $C_{22:6}$ (ω -3) in phosphatidylethanolamine was also apparent. Phosphatidylethanolamine, however, contained much more of the latter two fatty acids, particularly $C_{22:6}$ (ω -3), than phosphatidylcholine. Consequently the decrease in these ω -3 fatty acids was of greater magnitude in mitochondrial phosphatidylethanolamine. Through the longitudinal design employed in the present study it is apparent that dynamic turnover of phospholipid fatty-acyl chains occurs. This is clearly demonstrated in that the 11-day soya-bean oil to rapeseed oil cross-over period was of sufficient duration that by the end of this treatment period the fatty-acid composition of mitochondrial phosphatidylcholine and phosphatidylethanolamine resembled that of rats fed rapeseed oil consistently from day 0. On the basis of average values for the 11-day rapeseed oil cross-over phase neither $C_{22:4}$ nor $C_{22:5}$ (ω -6) fatty acids in phosphatidylcholine or phosphatidylethanolamine were fully suppressed, whereas the contents of $C_{20:1}$ in phosphatidylcholine and of $C_{18:1}$ and $C_{22:1}$ in phosphatidylethanolamine did not rise to the full extent present in rats fed rapeseed oil from day 0. By returning animals to soya-bean oil after the 11-day period of rapeseed oil feeding all fatty acids resumed values present in rats fed soya-bean oil from day 0, except for $C_{20:1}$ and $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$ fatty acids, the mean values of which were still somewhat increased in phosphatidylcholine and phosphatidylethanolamine respectively, and $C_{22:4}$, which was lower in phosphatidylethanolamine. Cardiolipin responded to the change in dietary oil type from soya-bean oil to rapeseed oil with an increase in $C_{18:1}$, $C_{20:1}$ and $C_{22:1}$ fatty acids and a decrease in $C_{18:2}$, $C_{22:3}$,

$C_{22:5}$ (ω -3) and $C_{22:6}$ fatty acids. Levels of these fatty acids became similar during days 12–23 (Fig. 1) to those of rats fed rapeseed oil from day 0, except that $C_{22:1}$ was somewhat lower on the cross-over treatment (Table 3). Changing back to soya-bean oil at day 23 resulted in return of $C_{18:1}$, $C_{22:5}$ (ω -3) and $C_{22:6}$ fatty acids to levels similar to those of cardiolipin from rats fed soya-bean oil from day 0. However, the mean value of $C_{22:3}$ remained decreased and $C_{20:1}$ and $C_{22:1}$ increased. No significant decrease in $C_{20:1}$ or $C_{22:1}$ occurred in cardiolipin during the 11-day soya-bean oil cross-back phase. This observation is in contrast with phosphatidylcholine and phosphatidylethanolamine, in which both $C_{20:1}$ and $C_{22:1}$ were significantly depleted by removal of these fatty acids from the diet for 11 days. Cross-over from rapeseed oil to soya-bean oil resulted in a decline of $C_{16:0}$ and $C_{18:0}$ content in cardiolipin below that of rats fed soya-bean oil only. In partial agreement with this observation data of Blomstrand & Svensson (1974) show elevation of saturated fatty acids in cardiolipin of cardiac mitochondria from rats fed rapeseed oil for 10 days. Elevation of saturated fatty acids in cardiolipin by rapeseed oil is in distinct contrast with the effects of this dietary fat on phosphatidylcholine and phosphatidylethanolamine. Increase in the relative proportion of saturated fatty acids in cardiolipin may not be fully evident in the present study (Table 3) due to a marked decline in total saturation of cardiolipin that occurs with increasing age inherent in the longitudinal design of the present study. These changes in saturation/unsaturation in cardiolipin may best be described as a decrease in saturated bonds concomitant to increase in U.I. and total ω -6 fatty acids induced by feeding diets high in polyunsaturated fatty acids. Relative proportions of fatty acids in cardiolipin other than those discussed above was not influenced by changing dietary oil from soya-bean oil to rapeseed oil or vice versa. The marked decreases of $C_{18:2}$ in cardiolipin and of $C_{22:6}$ in phosphatidylethanolamine observed from the 11-day cross from soya-bean oil to rapeseed oil substantiate a previous report (Blomstrand & Svensson, 1974) and were also observed by feeding rapeseed oil from 0 to 12 days (results not shown). After 12 days of rapeseed oil feeding values of both fatty acids had recovered to resemble contents present in rats fed soya-bean oil only.

Regression analyses for individual phospholipid fatty acids significantly influenced by dietary oil treatment are summarized (Table 4). The fatty acid composition of phosphatidylcholine, phosphatidylethanolamine and cardiolipin separated from inner membrane of heart mitochondria did not change between days 12 and 34 (i.e. the slope of the regression line was not significantly different from zero) for rats fed one dietary oil treatment with the

following exceptions: $C_{16:0}$, $C_{16:1}$, $C_{22:5}$ ($\omega-3$) in phosphatidylcholine; $C_{18:2}$, $C_{18:3}$ ($\omega-3$), $C_{22:5}$ ($\omega-3$) in phosphatidylethanolamine, $C_{18:2}$ in cardiolipin increased with time, whereas $C_{20:3}$ in phosphatidylcholine and phosphatidylethanolamine and $C_{20:0}$ and $C_{24:0}$ in phosphatidylethanolamine decreased with time, for both rats fed soya-bean oil or rapeseed oil. For rats fed rapeseed oil $C_{18:1}$ and $C_{18:2}$ in phosphatidylcholine, $C_{22:6}$ in phosphatidylethanolamine and $C_{22:1}$ in cardiolipin increased, whereas $C_{20:0}$ and $C_{20:2}$ in phosphatidylcholine, $C_{18:3}$ ($\omega-6$), $C_{20:1}$, $C_{22:4}$ and $C_{22:5}$ ($\omega-6$) in phosphatidylethanolamine and $C_{16:0}$ in cardiolipin decreased. In animals fed soya-bean oil, $C_{22:5}$ ($\omega-6$) in phosphatidylcholine and $C_{18:0}$ in cardiolipin decreased. The slope of the regression lines (i.e. rate of depletion or accretion) for individual fatty acids in phosphatidylcholine were similar between rats fed only soya-bean oil and rats fed only rapeseed oil for all fatty acids except $C_{18:2}$ and $C_{20:2}$. $C_{18:2}$ increased more rapidly with time in rapeseed oil after initial suppression of phosphatidylcholine $C_{18:2}$ values by rapeseed oil before day 12 (results not shown). $C_{20:2}$ decreased with time on rapeseed oil after its prior accumulation from day 0–12 in rats fed this oil. Rates of accretion and depletion for individual fatty-acyl chains of phosphatidylethanolamine were similar for rats fed soya-bean oil and rats fed rapeseed oil, except for a decrease in $C_{18:3}$ ($\omega-6$) of phosphatidylethanolamine after the initial increase of this fatty acid in phosphatidylethanolamine induced by rapeseed oil from day 0–12. Additionally the $C_{20:0}$ content of phosphatidylethanolamine declined more rapidly between days 12 and 34 in rats fed soya-bean oil than in rats fed rapeseed oil. The rates of accretion or depletion for individual fatty acids in cardiolipin between rats fed soya-bean oil and rats fed rapeseed oil were similar in all cases except for $C_{18:2}$ (discussed above). Values of $C_{22:1}$ in cardiolipin of rats fed rapeseed oil increased throughout the experiment. The persistent rise in $C_{22:1}$ in cardiolipin is in contrast with the apparent consistent value of this fatty acid in mitochondrial phosphatidylcholine and phosphatidylethanolamine after day 12 for rats maintained on rapeseed oil from day 0 to 34. Specific affinity of $C_{22:1}$ for the cardiolipin molecule has previously been proposed (Blomstrand & Svensson, 1974).

The difference in sign of the slopes for the regression lines representing the two phases of the cross-over (Table 4) indicate that by altering dietary oil composition dynamic replacement and repletion of membrane fatty acids can be effected. Regression lines constructed from these data (Table 4) clearly depict the type of changes achieved (examples in Fig. 2) and provide a model of the dynamic nature of fatty-acyl chains in membrane phospholipids

emphasizing the rapid onset of change via modulation of dietary fat composition. By changing from the polyunsaturated-fatty-acid-rich diet (soya-bean oil) to the monounsaturated-fatty-acid-rich diet (rapeseed oil) for 11 days, then returning to soya-bean oil for a further 11 days, depletion of $C_{16:0}$, $C_{18:0}$, $C_{18:2}$, $C_{22:4}$ and $C_{22:5}$ ($\omega-6$) in phosphatidylcholine and phosphatidylethanolamine and of $C_{22:6}$ in phosphatidylethanolamine followed by subsequent repletion of these fatty acids was achieved. $C_{18:2}$ in phosphatidylethanolamine was increased by the return to soya-bean oil, but in a non-linear manner (Table 3). Accretion of $C_{18:1}$, $C_{18:3}$ ($\omega-3$), $C_{20:0}$, $C_{20:1}$, $C_{20:2}$ and $C_{22:1}$ occurred in phosphatidylcholine and phosphatidylethanolamine during the rapeseed oil (days 12 to 23) cross-over followed by $C_{18:1}$, $C_{18:3}$ ($\omega-3$), $C_{20:0}$, $C_{20:1}$ and $C_{22:1}$ depletion in phosphatidylcholine and phosphatidylethanolamine and depletion of $C_{20:2}$ in phosphatidylcholine during the return to soya-bean oil (days 23 to 34). In cardiolipin changing from soya-bean oil to rapeseed oil and then from rapeseed oil to soya-bean oil resulted in depletion then repletion for $C_{18:2}$, $C_{22:5}$ ($\omega-3$) and $C_{22:6}$. Cardiolipin content of $C_{20:0}$, $C_{20:1}$ and $C_{22:1}$ increased during the soya-bean oil to rapeseed oil phase and subsequently declined during return to soya-bean oil. Values of $C_{18:1}$ in cardiolipin became elevated on the cross-over to rapeseed oil but were not significantly decreased by the return from rapeseed oil to soya-bean oil.

Effect of diet treatment on membrane phospholipid content

Feeding diets containing soya-bean oil or rapeseed oil and changing between these two oils had no apparent effect on total phospholipid or unesterified cholesterol content relative to protein content of the inner membrane of heart mitochondria. These observations confirm previous reports (Hung *et al.*, 1977; Christiansen *et al.*, 1979; Norseth, 1979) of failure to alter membrane total phospholipids via dietary manipulation. The phospholipid phosphorus/protein (approximate average for all groups from day 12, $13.84 \pm 0.53 \mu\text{g}$ of P_i /mg of protein) and unesterified cholesterol/protein (approximate average for all groups from day 12, $2.18 \pm 0.10 \mu\text{g}$ /mg of protein) found in the present experiments are similar to published values (Parsons & Yano, 1967) for the inner membrane of heart mitochondria. In the current experiments dietary oil induced an apparent change in the concentration of membrane phosphatidylcholine and phosphatidylethanolamine. The concentration of phosphatidylcholine (measured as μg of P_i /mg of protein) relative to the concentration of phosphatidylethanolamine (μg of P_i /mg of protein) was increased in rats fed rapeseed oil compared with rats fed soya-bean oil (ratio phosphatidyl-

ethanolamine/phosphatidylcholine from day 12: soya-bean oil, 1.22 ± 0.09 ; rapeseed oil, 0.85 ± 0.04 ; rapeseed oil cross-over, 0.87 ± 0.07 ; soya-bean oil cross-over, 1.01 ± 0.10). Dietary oil had no effect on cardiolipin concentration of the mitochondrial inner membrane (mean for all treatments from day 12, $1.91 \pm 0.09 \mu\text{g}$ of P_i/mg of protein). Results reported by Blomstrand & Svensson (1974) suggest a similar tendency towards increased concentration of phosphatidylcholine and decreased concentration of phosphatidylethanolamine in cardiac mitochondrial membranes of rats fed diets containing rapeseed oil in comparison with rats fed peanut oil.

Discussion

These studies clearly show the controlling influence of dietary long-chain fatty acids on the fatty-acyl components of rat heart mitochondrial inner membrane under conditions of adequate essential fatty-acid status. The rapid effect of changes in dietary fatty-acid composition or balance on mitochondrial phospholipid fatty acids suggest that such dietary influence is not unique to this organelle membrane and may, therefore, induce analogous membrane compositional changes in other cell types. Essential-fatty-acid deficient or limiting diets have been used almost exclusively to demonstrate extrinsic influence on phospholipid fatty acids of several membranes concomitant with alteration in membrane physical properties and activity of associated enzymes (Haeffner & Privett, 1975; King *et al.*, 1977; Hammer & Wills, 1979; Im *et al.*, 1979; Rao *et al.*, 1979). The observation that essential-fatty-acid-compromised diets promote changes in this lipid class, normally rich in $\omega-6$ and $\omega-3$ families, should not be unexpected. Results of the present study demonstrate a new physiological principle whereby in the whole animal membrane lipid is modulated by dietary long-chain fatty acids even when the diet is adequate in all nutrients. This principle further suggests (i) the basic concept of the necessity for dietary fatty-acid balance if consistency of optimal membrane structural lipid composition is to be maintained, as well as (ii) the overall inadequacy of describing the nutritional-biochemical quality of a dietary fat solely by its content of linoleic acid.

The importance of phospholipid fatty-acyl components as a major factor influencing physical properties of membranes and functioning of membrane-associated enzymes is well recognized. Positional distribution and composition of fatty acids in the membrane is subject to intrinsic controls. Non-random fatty-acid distribution results from acylation specificity during synthesis *de novo*, redistribution of fatty-acyl chains via phospholipase and acyltransferase (McMurray & Magee, 1972)

and by direct desaturation of membrane phospholipid-linked fatty acids (Pugh & Kates, 1979). These intrinsic mechanisms theoretically could provide and maintain optimal membrane composition despite fluctuations in dietary lipid. The studies presented herein, however, demonstrate the membrane lipid is also susceptible to extrinsic control. Thus it is not unreasonable to speculate that physicochemical properties of membranes are subjected to continuous subtle changes with variation in lipid intake in the growing animal.

The dynamic nature of membrane fatty-acyl chains is readily apparent from the present study. Maximal influence on the majority of fatty acids was achieved in the 11-day cross-over periods. Thus, substituting rapeseed oil at a level of 20% (w/w) of the diet for soya-bean oil at a similar concentration resulted in a shift in composition of mitochondrial inner membrane phosphatidylcholine, phosphatidylethanolamine and cardiolipin towards that of animals fed rapeseed oil continually. By returning to soya-bean oil these effects were essentially reversed (Tables 2 and 3). Such rapid changes in membrane lipid composition fit that predicted from reported short half-lives of the fatty-acyl components of phospholipids, which exhibit turnover independently of the entire phospholipid molecule (Landriscina *et al.*, 1976). Results obtained as a result of the longitudinal design of the present study also demonstrate that changes in fatty-acid composition of phospholipids (for example, increase of $\text{C}_{18:2}$ in cardiolipin; Fig. 2b) normally occur with growth in the weanling animal.

The similarity of the influence of dietary fat composition on the response of mitochondrial phosphatidylcholine and phosphatidylethanolamine as distinct from that of cardiolipin is noteworthy, particularly in view of other biosynthetic differences that exist between these phospholipids. Phosphatidylcholine and phosphatidylethanolamine are synthesized on the endoplasmic reticulum whereas cardiolipin is exclusively of mitochondrial origin (Holub & Kuksis, 1978). Conceivably, differences exist in fatty-acid pools available between these cellular sites at the time of phospholipid synthesis *de novo*. Fatty acids in phosphatidylcholine and phosphatidylethanolamine show positional specificity with preferential localization of saturated fatty acids at the 1-position and unsaturated fatty acids at the 2-position (Holub & Kuksis, 1978), whereas fatty acids in cardiolipin are randomly distributed between the 1- and 2-position (Marinetti, 1964). Such positional specificity, together with differences in preferred fatty-acid composition normally occurring among phospholipid classes (Holub & Kuksis, 1978), may determine the extent of influence of a given dietary lipid on individual phospholipid classes. Additionally, the asymmetric distribution of

phospholipids across the mitochondrial inner membrane (Crain & Marinetti, 1979; Krebs *et al.*, 1979) may involve pools of differing fatty-acid composition for participation in deacylation-reacylation reactions. Turnover of fatty acids in cardiolipin is slower than in other phospholipids (Table 3; Landriscina *et al.*, 1976). The response of cardiolipin to dietary lipid change in the present study, particularly with regard to the monoenoic fatty acids of rapeseed oil, appeared to be slower than for phosphatidylcholine and phosphatidylethanolamine, perhaps as a result of longer-fatty-acid half-life and difference in asymmetric distribution of cardiolipin within the mitochondrial inner membrane.

Explanation for apparent changes in inner-membrane content of phosphatidylcholine/phosphatidylethanolamine, but not of cardiolipin, as a result of dietary fat is not obvious and requires further study. A change in polar-head-group distribution in the inner membrane could have marked influences on membrane properties and functions. It is worth pointing out that alteration in phospholipid distribution may have resulted either from a direct effect of a specific oil or oil component on phospholipid synthesis and/or turnover, or it may be a compensatory measure evoked by the animal in response to altered membrane fatty-acyl composition. Results reported by Kramer *et al.* (1979) show increased phosphatidylcholine (mg/g of heart) and decreased phosphatidylethanolamine (mg/g of heart) after 16 weeks of 20% (w/w) dietary rapeseed oil treatment in rats of the Sprague-Dawley strain in comparison with rats of the Chester Beatty strain. At this time (16 weeks) the number and severity of rapeseed oil-induced heart lesions in the Chester Beatty rat is much lower than in the Sprague-Dawley rat (Kramer *et al.*, 1979). Possibly a relationship exists between rapeseed oil-induced alteration in mitochondrial inner-membrane phospholipid distribution in addition to fatty-acyl-chain compositional changes (Tables 2 and 3) and the onset of changes in mitochondrial ultrastructure (Yamashiro & Clandinin, 1980) and activity of inner-membrane-associated enzymes (Clandinin, 1978, 1979; Renner *et al.*, 1979).

The study reported herein uniquely demonstrates that modification of subcellular membranes can be controlled in a reversible manner via manipulation of dietary fatty-acid balance in an intact growing animal, thus suggesting that membrane-associated metabolic functions in the cell may be controlled in a similar manner. The results establish the dynamic nature of membrane lipids in the mammalian system with a novel experimental design that has broad application for investigation of other membrane systems *in vivo*.

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