Comparative Studies on Composition of Cardiac Phospholipids in Rats Fed Different Vegetable Oils[1]

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ABSTRACT

Male Sprague-Dawley rats were fed diets for 1 or 16 weeks, containing 20% by weight vegetable oils differing widely in their oleic, linoleic and linolenic acid content. No significant changes were observed in the level of the cardiac lipid classes. The fatty acid composition of the 2 major phospholipids, phosphatidylcholine and phosphatidylethanolamine, showed a remarkable similarity between diets in the concentration of total saturated, C22 polyunsaturated and arachidonic acids. Monounsaturated acids were incorporated depending on their dietary concentration, but the increases were moderate. Dietary linolenic acid rapidly substituted C22 polyunsaturated fatty acids of the linoleic acid family (n-6) with those from the linolenic acid family (n-3). The results suggest that dietary linolenic acid of less than 15% does not inhibit the conversion of linoleic to arachidonic acid but the subsequent conversion of arachidonic acid to the C22 polyunsaturates was greatly reduced. Significant amounts of dietary monounsaturated fatty acids were incorporated into cardiac cardiolipin accompanied by increases in polyunsaturated fatty acids, apparently to maintain an average of 2 double bonds/molecule. The cardiac sphingomyelins also accumulated monounsaturated fatty acids depending on the dietary concentration. It is quite evident from the results of this study that the incorporation of oleic acid and the substitution of linolenic for linoleic acid-derived C22 polyunsaturated fatty acids into cardiac phospholipids was related to the dietary concentration of these fatty acids and was not peculiar to any specific oil. Even though it is impossible to estimate the effect of such changes in cardiac phospholipids on membrane structure and function, results are discussed which suggest that the resultant membrane in the Sprague-Dawley male rat is more fragile, leading to greater cellular breakdown and focal necrosis.

INTRODUCTION

There is extensive evidence to indicate that myocardial lesions in male albino rats fed diets rich in fat or vegetable oil do not result from cardiopathogenic compounds in these fats and oils (1-4) but are related to several dietary fatty acids (5). Linolenic (18:3n-3) (5-8), oleic (18:1n-9) (7,8) and erucic (22:1n-9) acids (6,9) at high levels have been implicated in the etiology of cardiac necrosis, whereas saturated fatty acids (5,10) and linoleic (18:2n-6) acid (7,8) apparently are related to a lower incidence and severity of myocardial lesions in albino male rats.

Dietary fatty acids are known to influence the fatty acid composition of tissue lipids (11). Of several organs tested in the rat, the heart was shown to be most responsive to changes in long-chain polyunsaturated fatty acids (PUFA) when the rats were fed either 18:2n-6 or 18:3n-3 (12). Since these long-chain PUFA are found mainly in phospholipids which are membrane constituents (13), changes in the PUFA may have important consequences in membrane properties and function. These changes, in turn, may be related to the myocardial muscle damage seen in male rats.

In this study, different vegetable oils were chosen to provide a range of dietary fatty acids (i.e., 18:1, 18:2n-6 and 18:3n-3) similar to those found in low erucic acid rapeseed (LEAR) oils, in order to investigate whether the cardiac phospholipid changes in rats fed LEAR oils are peculiar to LEAR oils or simply reflect the dietary fatty acids irrespective of source. Previous studies failed to include control oils that contained similar levels of 18:1 (14), 18:2n-6 (15) and 18:3n-3 (14-19) found in LEAR oils and therefore could not adequately compare the effect of all these dietary fatty acids. In addition, the effects of dietary oils within the first week on the fatty acid composition of the major cardiac phospholipids in the rat were investigated.

MATERIALS AND METHODS

Animals and Diets

Weanling male Sprague-Dawley rats, 3 weeks of age, were supplied by Bio-Breeding, Ottawa, Ontario, and weighed between 40 and 50 g. The rats were distributed randomly to 5 dietary treatments, 5 rats/treatment, and fed ad libitum the test diets for 16 weeks. An additional 10 rats, 5/diet, were fed a diet containing corn oil or LEAR (cv. Zephyr) for 1 week. Five rats were killed immediately after weanling. Water

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was available at all times. The semisynthetic diets, described previously (20), contained 20% by weight one of the following oils: corn, olive, soybean, *Brassica napus* cv. Tower and *B. napus* cv. Zephyr.

Extraction and Analysis of Lipids

Rats were anesthesized with CO_2 and decapitated. The hearts were removed immediately, weighed, frozen between 2 blocks of dry ice, then pulverized and the lipids extracted as described previously (21). Total lipids were determined gravimetrically.

An Iatroscan TH-10 TLC Analyzer, Mark II (Iatron Laboratories, Inc., Tokyo, Japan; Canadian distributer: Technical Marketing Associates, Ltd., Mississauga, Ontario) was used to determine the relative composition of the cardiac lipid classes. The instrument was equipped with flame ionization detector (H_2 flow rate, 175 ml/min; air flow rate, 1850 ml/min), scanner (scanning speed, 0.47 cm/sec), integrator and recorder (sensitivity, 10 mV; chart speed, 0.42 cm/sec). A package of 10 chromarods (silica rods with a sintered coating of active adsorbent mixed with glass powder, mean thickness 75 μm) was soaked overnight in 9 N H_2SO_4. The rods were then rinsed with distilled water, dried at 100 C and prescanned twice before use. About 1 μl of total cardiac lipids dissolved in $CHCl_3/CH_3OH$ (2/1) was spotted on the rods. The rods were successively developed for a distance of 10 cm using the following solvents: (a) hexane/diethyl ether/formic acid (85:15:0.04), (b) acetone, and (c) $CHCl_3CH_3OH/H_2O$ (67:29:4). After each development, the rods were placed in an oven at 90 C for 5 min and then scanned. The rods were only partially burned from R_f 1.0 to ca. R_f 0.25 following the first and second development, and completely burned after the final development. After the first burn, mono- and diglycerides, cholesterol and the phospholipids remained; after the second burn, the phospholipids were not burned. The triglycerides had a response factor of 0.65 compared to the other lipid classes and therefore a correction factor of 1.5 was applied. The correction factors for most major components was close to unity as has been reported previously (22-24).

Total cardiac lipids (ca. 3 mg) were separated by 2-dimensional thin layer chromatography (TLC) using the solvents described by Rouser et al. (25). TLC plates were dried under N_2. Spots were visualized under ultraviolet (UV) light after spraying the plate with 2'7'-dichlorofluorescein, and scraped off the plate directly into 15-ml screw-capped tubes. Methyl esters were prepared by transesterification (21), purified on TLC and analyzed by a Hewlett Packard Model 5830 gas chromatograph, using glass columns (1.8 m x 2 mm) packed with 5% SP-2310 on 100/120 Chromosorb W AW (Supelco Inc., Bellefonte, PA). The alkenyl ethers were analyzed as described previously (26).

Analysis of variance was applied to all data. The least significant differences at the 1% level were determined from the error estimates (27).

RESULTS

Dietary oils were chosen to evaluate the effect of several fatty acids found in LEAR oils on the cardiac lipids of male rats. Soybean oil provided a similar concentration of 18:3n-3 and olive oil contained high levels of 18:1 (Table I). Corn oil was selected as an oil rich in 18:2n-6.

Male rats fed the diet containing corn oil for 16 weeks showed the best growth, whereas rats fed the other dietary oils were slightly lower (Table II). The dietary oils apparently had little effect on either the heart weight or on the total cardiac lipids, except for rats fed LEAR (cv. Zephyr) which had the lowest weight of lipid/g wet heart.

TABLE I

Fatty Acid Composition of Dietary Oils

Fatty acids	Corn	Olive	Soybean	Tower[a]	Zephyr[b]
16:0	10:9	11.6	12.4	6.1	5.3
18:0	1.7	2.5	3.7	2.0	2.3
18:1	24.3	75.5	25.4	56.5	64.7
18:2n-6	61.1	7.3	50.6	26.0	17.5
18:3n-3	0.9	0.7	7.9	7.1	5.6
20:1	0.2	0.4	-	1.5	1.5
22:1	-	0.1	-	0.3	0.9

[a]*Brassica napus* cv. Tower, a rapeseed oil low in erucic acid and glucosinolates.

[b]*Brassica napus* cv. Zephyr, a rapeseed oil low in erucic acid.

TABLE II

Body, Heart and Heart Lipid Weights, and Relative Composition of Heart Lipid

Description	Diets[a]					LSD[b]
	Corn	Olive	Soybean	Tower	Zephyr	
Body wt (g)	514	493	482	483	483	27.8
Heart wt (g)	1.34	1.25	1.27	1.35	1.39	0.12
Lipid wt (mg/g heart)	29.57	32.21	29.84	30.86	26.99	4.2
Lipid classes[c] (relative %)						
CE	1.5	1.5	2.1	1.8	1.8	0.8
TG	20.9	22.6	19.4	23.7	23.2	5.8
C	6.1	5.6	5.9	5.4	5.8	1.3
CL	8.9	8.4	8.0	7.9	7.9	1.0
PE	21.0	20.1	21.6	20.2	18.9	3.7
PS and PI	2.2	2.6	2.3	2.8	3.1	1.2
PC	34.5	33.3	34.7	32.4	32.8	4.2
SP	3.2	4.2	3.9	3.9	4.3	1.7
LPC	1.3	1.3	1.8	1.3	1.6	0.5

[a]All values are mean of 5 rats per diet, except body weight which represents the mean of 50 rats per treatment (20).

[b]LSD = least significant difference obtained from pooled error estimates of analysis of variance. Means within a row differing by more than the LSD are significantly different at the 1% level.

[c]Abbreviations: cholesterol ester (CE), triglyceride (TG), cholesterol (C), cardiolipin (CL), phosphatidylethanolamine (PE), -serine (PS), -inositol (PI) and -choline (PC), sphingomyelin (SP), and lysophosphatidylcholine (LPC).

A total analysis of the cardiac lipid subclasses was achieved with an Iatroscan using 3 separate solvent systems followed by a partial combustion technique between developments. The results are shown in Table II. There were no significant differences ($P > 0.01$) between diets in any of the lipid classes of the heart. However, diets rich in 18:1 (olive and LEAR oils) were associated with slightly higher levels of triglycerides and somewhat lower concentrations of PE and PC compared to rats fed soybean and corn oil.

The fatty acid compositions of the 2 major phospholipids of weanling rats and those fed the experimental diets for 1 and 16 weeks are given in Table III. There apparently was no effect of diet fed and age of rat on the amount (mg/g wet heart) of phosphatidylethanolamine (PE) and phosphatidylcholine (PC). Furthermore, there apparently was little effect of diet on the level of total saturated fatty acids and dimethylacetals (DMA) derived from the plasmalogenic (alkenyl ethers) compounds in these cardiac phospholipids. The concentration of monoenoic fatty acids increased significantly in rats fed diets rich in 18:1; rats fed olive oil and LEAR oil were similar. This change was rapid, i.e., after 1 week the concentration of 18:1 in cardiac PE and PC of the rat fed Zephyr oil already resembled that of the rat fed for 16 weeks.

The total level of PUFA in cardiac PE was surprisingly similar between the diets fed and time periods investigated (Table III). The major PUFA, arachidonic acid (20:4n-6), also remained fairly constant. The PUFA of PE of weanling rats were high in n-3 family acids. Dietary 18:2n-6, with little 18:3n-3 present (i.e., < 1%), rapidly substituted n-6 for n-3 PUFA, whereas a dietary level of more than 5% 18:3n-3 retained or increased the level of n-3 PUFA. Nowhere was this more evident that in the PE C22 PUFA of rats fed corn and Zephyr (Fig. 1). Although the sum of all C22 PUFA was similar between the 2 diets at both 1 and 16 weeks, the rats fed Zephyr oil were practically devoid of n-6 C22 PUFA after 16 weeks, whereas the corn-oil-fed rats had markedly reduced n-3 C22 PUFA.

The total level of PUFA in cardiac PC was slightly higher in rats fed diets rich in 18:2n-6 (corn and soybean oils) than in rats fed olive or LEAR oils (Table III). Arachidonic acid was slightly lower in rats fed LEAR oils than in rats fed corn or soybean oil, but the rats fed olive oil, with the lowest dietary level of 18:2n-6, had the highest level of 20:4n-6 and rather low levels of 18:2n-6. The C22 PUFA in cardiac PC were considerably lower than in cardiac PE. However, as in PE, the sum of the C22 PUFA was similar between the age of rats and the diets fed, and depending on the dietary level of 18:2n-6 and 18:3n-3, n-6 or n-3 C22 PUFA predominated (Fig. 1).

Linoleic acid was the major fatty acid in cardiac cardiolipin (Table IV), and the relative

TABLE III

Fatty Acid Composition of Cardiac Phosphatidylethanolamine and Phosphatidylcholine from Weanling Rats and Rats Fed Diets Containing Vegetable Oils for 1 and 16 weeks

	Phosphatidylethanolamine									Phosphatidylcholine								
		1 Week		16 Weeks							1 Week		16 Weeks					
Fatty acids[a]	0	Corn	Zephyr	Corn	Olive	Soybean	Tower	Zephyr	LSD[b]	0	Corn	Zephyr	Corn	Olive	Soybean	Tower	Zephyr	LSD
16:0 DMA[a]	5.8[d]	4.5	3.7	4.3	3.9	4.3	3.2	3.5	0.7	0.7	0.5	0.5	0.5	0.5	0.6	0.4	0.4	0.1
16:0	8.0	5.8	3.5	6.2	5.4	5.9	5.0	4.4	1.2	18.4	16.7	11.9	13.2	12.9	12.7	12.8	11.2	1.4
18:0 DMA	3.4	4.0	2.4	2.5	2.3	3.5	2.2	2.3	0.5	0.2	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.1
18:0	23.9	24.2	24.9	24.5	26.7	26.1	26.6	25.8	1.0	24.3	23.8	23.2	26.9	27.3	28.7	26.4	26.8	1.2
18:1	4.9	5.8	11.4	6.6	9.5	5.3	8.9	10.9	1.5	9.4	7.9	16.8	7.0	13.1	7.0	12.2	13.8	0.9
18:2 n-6[e]	4.3	8.0	6.2	9.4	2.3	6.7	6.0	4.9	1.8	12.0	16.5	14.8	13.3	3.9	10.5	10.5	10.0	2.8
18:3 n-3	0.2	0.1	0.6	-	-	0.3	0.4	0.3	0.1	0.1	tr	0.6	-	-	0.3	0.3	0.3	0.1
20:1	0.4	0.4	0.8	0.3	0.2	0.2	0.4	0.3	0.1	0.4	0.4	0.8	0.2	0.3	0.2	0.4	0.4	0.1
20:4 n-6	22.6	22.5	22.7	20.4	26.5	17.9	19.7	22.1	2.8	24.6	25.9	22.4	31.0	34.3	31.0	27.9	29.5	1.9
22:1	0.1	-	0.2	-	-	-	0.1	0.1	0.05	0.1	-	0.3	-	-	-	0.1	0.2	0.04
22:4 n-6	2.6	3.8	1.6	3.4	1.0	1.3	0.9	0.9	0.5	1.3	1.7	0.6	1.5	0.5	0.7	0.4	0.4	0.3
22:5 n-6	2.5	7.8	1.7	13.1	5.3	1.3	0.5	0.3	1.8	0.5	1.9	0.3	3.0	1.3	0.3	0.2	0.1	0.5
22:5 n-3	3.1	1.4	2.4	0.7	0.5	1.8	2.2	2.1	0.6	2.1	0.8	1.4	0.3	0.3	1.3	1.4	1.2	0.5
22:6 n-3	15.7	9.1	15.0	6.7	15.0	24.1	22.8	20.6	3.7	3.8	1.8	3.5	1.5	3.6	5.8	5.8	4.6	1.7
mg/g wet weight	5.3	5.6	5.1	6.2	6.5	6.4	6.2	5.1	2.3	8.9	9.5	9.2	10.2	10.7	10.4	10.0	8.9	3.4
DMA	9.2	8.5	6.1	6.8	6.2	7.8	5.4	5.8	1.1	0.9	0.7	0.7	0.8	0.8	0.8	0.6	0.6	0.2
Σ Saturates	32.7	31.2	29.4	31.4	32.4	32.3	32.1	30.6	1.4	43.7	41.5	35.8	40.5	40.4	41.5	39.5	38.2	0.8
Σ Monounsaturates	6.5	7.2	13.5	7.1	10.0	5.9	9.8	11.9	1.5	9.9	8.3	18.5	7.2	13.4	7.2	12.7	14.2	0.9
Σ Polyunsaturates	51.6	53.1	51.0	54.4	51.0	53.5	52.6	51.3	1.0	45.5	49.5	45.0	50.9	44.7	50.1	46.5	46.1	0.8
Σ n-6	32.6	42.6	33.0	47.0	35.5	27.3	27.2	28.3	3.6	39.3	46.9	39.1	49.1	40.8	43.0	39.2	40.3	2.0
Σ n-3	19.0	10.6	18.0	7.4	15.5	26.2	25.4	23.0	3.7	6.2	2.6	5.9	1.8	3.9	7.1	7.2	5.8	1.8
Σ C22 PUFA[f]	23.9	22.1	20.7	23.9	21.8	28.5	26.4	23.9	4.5	7.7	6.2	5.8	6.3	5.7	8.1	7.8	6.3	2.2

[a]Minor amounts of 14:0, 15:0, 17:0, 20:0, 20:2 and 20:3 are not included in the table.

[b]LSD = least significant difference at the 1% level (see footnote b Table II).

[c]DMA = dimethylacetal. These compounds are derived from alkenyl ethers during methylation.

[d]All values are the mean of 5 rats per diet. tr = trace (< 0.05%); (-) = no detectable amount.

[e]n-x, where n is the chain length of the fatty acids and x the number of carbon atoms from the last double bond to the terminal methyl end of the molecule.

[f]PUFA = polyunsaturated fatty acid.

concentration of this acid increased significantly compared to weanling rats on all diets except olive oil. Rats fed olive oil accumulated excessive amounts of 18:1, which were accompanied by PUFA of both the n-6 (20:4n-6 and 22:5n-6) and n-3 (22:6n-3) families to retain an average number of 2 double bonds (sum of the percentage of individual unsaturated fatty acids X number of double bonds/100) for cardiac cardiolipin. Weanling rats also contained substantial amounts of 20:4n-6 and 22:6n-3 in their cardiolipin fraction to maintain an average number of 2 double bonds. The effect of dietary 18:3n-3 was relatively minor; rats fed soybean and LEAR oil contained similar levels of n-3 PUFA.

There apparently was little difference between diets in the abundance of DMA produced during methylation (Table III). The composition of the alkenyl groups of 2 selected diets, soybean and Tower, are shown in Table V. Dietary oleic and linoleic acids influenced the alkenyl group composition; no alkenyl chains derived from linolenic or erucic acids were detected. The alkenyl group composition of the 2 phosphoglycerides was similar.

The fatty acid composition of sphingomyelin is shown in Table VI. The main difference between diets occurred with respect to the monounsaturated acids. Rats fed LEAR oils or olive oil had significantly higher levels of total monounsaturates than rats fed corn or soybean oils. The 22:1 fatty acid was found in sphingomyelin of every dietary group, but the concentration of 22:1 was significantly higher in rats fed LEAR (cv. Zephyr) than in the other groups. The position of the double bond is presumably n-9, since the long-chain monoenoic acids of sphingomyelin are formed by chain elongation from oleic (18:1n-9) acid (29).

DISCUSSION

The cardiac lipids of male rats fed LEAR oils have been investigated in the past 10 years to

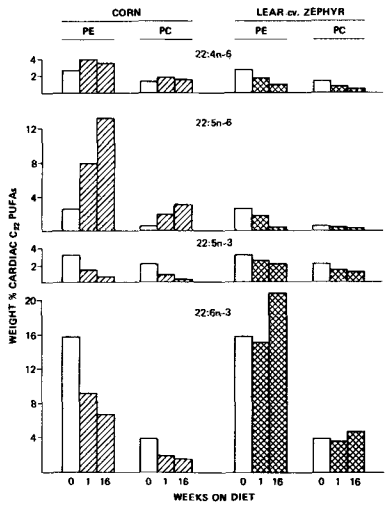

FIG. 1. The relative concentration of the C22 PUFA of cardiac phosphatidylethanolamine (PE) and phosphatidylcholine (PC) are compared in weanling rats (open bars) with rats fed diets containing corn oil or LEAR oil cv. Zephyr for 1 and 16 weeks.

TABLE IV

Fatty Acid Composition of Cardiac Cardiolipin from Weanling Rats and Rats Fed the Experimental Diets for 1 and 16 weeks[a]

Fatty acids	Weanling	1 Week		16 Weeks					LSD
		Corn	Zephyr	Corn	Olive	Soybean	Tower	Zephyr	
16:0	4.6	2.7	2.5	2.2	2.6	1.4	1.2	1.1	0.5
18:0	3.8	3.2	2.7	1.7	2.3	1.3	0.9	0.7	0.5
18:1	10.3	8.0	12.3	4.7	22.4	4.3	7.9	9.8	2.3
18:2n-6	56.4	72.9	66.9	85.0	51.7	85.8	81.7	79.1	3.2
18:3n-3	0.5	0.1	2.3	-	-	1.3	1.6	1.7	0.2
20:1	0.8	0.3	0.7	0.1	0.5	0.1	0.4	0.3	0.1
20:4n-6	6.7	4.0	3.7	1.9	7.5	1.8	1.6	1.7	0.4
22:1	-	-	0.2	-	-	-	tr	tr	-
22:4n-6	0.7	0.8	0.5	0.3	0.2	0.1	0.1	0.1	0.1
22:5n-6	1.1	0.9	0.4	0.9	2.5	0.1	tr	tr	0.1
22:5n-3	1.3	0.7	0.8	0.1	0.2	0.3	0.3	0.3	0.2
22:6n-3	5.7	1.8	2.5	0.4	5.2	1.5	1.5	1.8	0.7
Av. no of double bonds[b]	2.15	1.99	1.99	1.91	2.02	1.98	1.94	1.94	

[a]See footnotes to Table III.

[b]Average number of double bonds is the sum of the percentage of individual unsaturated fatty acids X number of double bonds/100.

TABLE V

Composition of the Alkenyl Groups from Rat Heart Ethanolamine and Choline Phosphoglycerides

Chain length: no. of double bonds	Ethanolamine phosphoglycerides		Choline phosphoglycerides	
	Soybean	Tower	Soybean	Tower
14:0	2.0	1.6	3.0	2.9
15:0	2.2	1.1	2.6	3.3
16:0	24.2	24.4	20.8	14.4
16:1	2.2	2.1	2.5	3.1
17:0	2.7	1.4	3.2	3.9
17:1	1.2	1.1	1.3	2.0
18:0	36.6	35.9	38.8	36.3
18:1	13.5	23.0	18.0	26.2
18:2	14.8	8.8	9.8	7.8

TABLE VI

Fatty Acid Composition of Sphingomyelin from Hearts of Rats Fed Vegetable Oils for 16 Weeks

Fatty acids	Diets[a]					LSD[b]
	Corn	Olive	Soybean	Tower	Zephyr	
14:0	0.5	0.6	0.8	0.9	1.0	0.5
15:0	0.4	0.5	0.5	0.7	0.5	0.3
16:0	13.4	13.1	12.5	11.4	14.5	2.8
17:0	0.6	1.1	0.7	0.8	0.6	0.6
18:0	11.5	12.4	12.6	12.5	14.3	2.2
18:1	1.6	2.9	1.4	2.1	2.6	1.6
19:0	1.7	1.2	1.0	1.9	1.5	0.7
20:0	24.9	19.7	15.9	21.0	20.0	2.3
21:0	1.3	1.6	2.0	0.9	0.9	0.3
22:0	16.1	15.7	26.4	21.6	17.6	2.8
22:1	0.2	0.5	0.4	0.5	0.9	0.3
23:0	4.5	4.6	5.2	2.5	2.4	0.6
ECL 23.6[c]	2.9	2.8	2.9	1.3	1.5	0.9
24:0	13.9	10.7	12.1	8.6	8.4	1.6
24:1	5.9	11.2	5.8	12.3	12.5	2.0
X:1[d]	7.7	14.6	7.6	14.9	16.0	

[a]Values are means of 5 rats per diet.

[b]LSD = least significant differences at the 1% level.

[c]ECL = equivalent chain length (28).

[d]X:1, monounsaturated fatty acids.

determine whether myocardial damage in rats fed dietary LEAR oils is related to changes in lipid classes or their fatty acid composition. Cardiac neutral lipids, particularly the triglycerides, were altered readily both qualitatively and quantitatively with different dietary fatty acids (30). However, these triglyceride changes have been discounted as the cause of the necrotic heart lesions as first assumed (31). The reason was that female rats of the same strain (Sprague-Dawley) (32) and male rats of another strain (Chester Beatty) (33) had similar cardiac triglyceride changes without causing the subsequent long-term necrotic heart lesions (32,33). Furthermore, the cardiac triglyceride accumulation was attributed to the dietary C22 long-chain monoenoic fatty acids (9,32).

The cardiac phospholipids, on the other hand, being mainly membrane constituents (13), have been found to be relatively resistant to quantitative changes; no changes had been observed in the total level of cardiac phosphorus from dietary oils (34). The results of this study demonstrated an in vivo regulation in the concentration of all cardiac phospholipids irrespective of the age of the rat (weanling 3-week-old, 1 and 16 weeks on diet) and source

of dietary oils. Only a slightly lower level of PE was noted in rats fed one of the 2 LEAR oils (cv. Zephyr). Blomstrand and Svensson (17) also observed decreased values of cardiac PE in rats fed diets containing low levels of erucic acid. No significant differences were observed in the level of sphingomyelin between rats fed LEAR oils or any other vegetable oil, similar to that reported by Dewailly et al. (18), and contrary to that reported by Beare-Rogers (35,36). The concentration of lyso-PC was low (1.8-2.5% recalculated as relative % of total phospholipids from Table II), and only traces of free fatty acids, and mono- and diglycerides were detected, indicating little, if any, autolysis of cardiac lipids during the extraction procedure employing an improved extraction technique (21). This was in marked contrast to lyso-PC values of 5-10% (% of total phospholipids) reported by Beare-Rogers et al. (14) using conventional extraction techniques. Extraction of cardiac lipids from rats fed high-fat diets are particularly susceptible to autolysis during conventional homogenizations, giving rise to high values of free fatty acids and lyso-PC (21).

As noted in the results of this study, there also was an apparent in vivo regulation in the fatty acid composition of all major cardiac phospholipids. In PE and PC, the total level of saturates and polyunsaturates remained fairly constant irrespective of the age of the rat and the dietary oil fed; the monounsaturates showed evidence of a linear relationship to dietary 18:1 ($r \geqslant 0.87$). Among the polyunsaturates, there was little change in 20:4n-6 and the total C22 PUFA. However, among the C22 PUFA, there were wide differences between n-3 and n-6 fatty acids, depending on the dietary concentration of 18:3n-3 or 18:2n-6, or including members of these fatty acids families as may be the case in weanling rats (37).

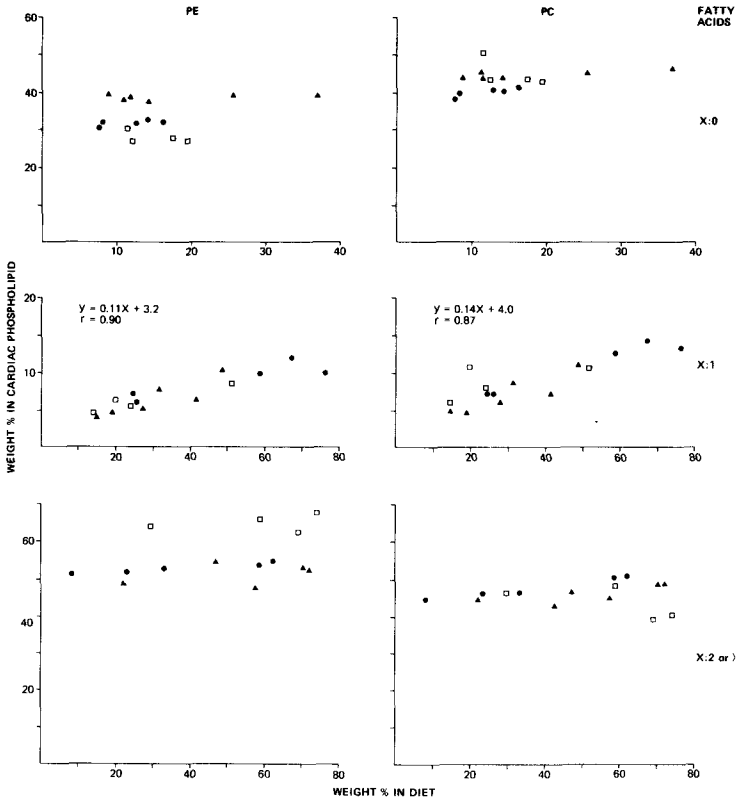

FIG. 2. The results of this study (•) were combined with those of 2 other publications (14 [▲] and 38 [□]). The relative concentration of dietary saturated (X:0), monounsaturated (X:1) and polyunsaturated (X:2 or >) fatty acids (abscissa) were plotted against the corresponding levels of these fatty acids in cardiac phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (ordinate). The relationship of monounsaturated fatty acids is represented by the linear equation, $y = ax + b$, where, a is the slope of the line, b the intercept, and x and y the concentration of monounsaturates in the diet and phospholipids, respectively. The correlation coefficient is r.

The results of this study were combined with those of 2 other studies (14,38) in which male rats were fed high-fat diets and cardiac PE and PC were analyzed to provide an even wider range of dietary fatty acids. The results are plotted in Figure 2. Dietary saturates (X:0), ranging from 8 to 37%, apparently had no effect on the concentration of saturates in cardiac PE or PC. On the other hand, a 5-fold difference in the dietary level of monounsaturates (X:1), from 14 to 76%, resulted in only a doubling of this group of fatty acids in either phospholipid. The biochemical mechanism of excluding monounsaturates from cardiac PE and PC may explain why 20:1 and 22:1 were not incorporated extensively into the 2 cardiac phospholipids, even when diets rich in these fatty acids were fed to rats (33). Finally, the total level of polyunsaturates (X:2 or >) remained fairly constant despite widely different levels of dietary 18:2n-6 (7-73%) and 18:3n-3 (trace to 53%). Among the PUFA, there was a small response of cardiac levels of 18:2n-6 to dietary 18:2n-6 (PE, $y = 0.13x + 6.3$, $r = 0.29$; PC, $y = 0.09x + 5.4$, $r = 0.64$). The concentration of 20:4n-6 remained fairly constant in PE and PC. Dietary levels of 5-14% 18:3n-3 depressed 20:4n-6 only slightly in PC, and it required a high dietary level of 18:3n-3 (52.6% in linseed oil) to depress 20:4n-6 significantly in both PE and PC.

The greatest change, however, was seen among the C22 PUFA (Fig. 3). In rats fed as little as 5% dietary 18:3n-3, most of the C22 PUFA consisted of the n-3 family, whereas the C22 PUFA of the n-6 family predominated in diets poor in 18:3n-3. The change in the C22 PUFA with diet were already evident after 1 week on the selected diets, corn and LEAR (cv. Zephyr) oils. The C22 PUFA of the n-3 family also predominated in weanling rats because the n-3 family acids are bioconcentrated in the fetus during its development (37). In addition, it suggests that the diet used by the supplier may have been rich in n-3 family acids. The results of Gudbjarnason et al. (39,40) and Rocquelin (15) show a similar replacement of n-3 for n-6 family acids in the total cardiac phospholipids by feeding diets containing 18:3n-3 (linseed oil, ref. 15) or n-3 family acids (cod liver oil, ref. 39).

Therefore, according to the results, dietary 18:3n-3 up to 15% apparently did not inhibit the conversion of 18:2n-6 to 20:4n-6, but the subsequent conversion to 22:4n-6 and 22:5n-6 was much reduced in favor of desaturation and elongation of 18:3n-3. Such a protective mechanism of maintaining a certain proportion of 20:4n-6 has also been observed in erythrocytes of premature infants (41).

Regulation of the fatty acid composition was also evident in cardiac cardiolipins, a component present in mitochondrial membranes (13). The substitution of monounsaturates in cardiolipin of rats fed olive oil apparently has been compensated for by the incorporation of PUFA to maintain a similar average number of double bonds of ca. 2. Monounsaturates have an apparent affinity for cardiac cardiolipin as demonstrated previously by Blomstrand and Svensson regarding 22:1n-9 (17).

It is quite evident from the results of this study that the incorporation of 18:1 and the substitution of n-3 for n-6 C22 PUFA into cardiac phospholipids was related to the dietary concentration of these fatty acids, and was not peculiar to any specific oil. At the present time, it is impossible to estimate the effect of these changes in cardiac phospholipids on the membrane structure and function, although it is known that specific phospholipids, and often certain fatty acids in these phospholipids, are required for full activity of several membrane-bound enzymes (13,37).

Evidence from this laboratory supports the concept that rats fed high-fat diets, particularly those rich in 18:3n-3, developed cardiac membranes which were "more fragile" than those

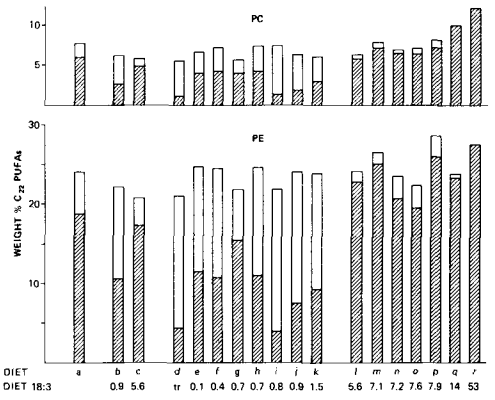

FIG. 3. The relative concentration of the n-3 (solid bar) and n-6 (open bar) C22 PUFA of weanling rats (a) and rats fed different dietary fats and oils for 1 (b and c) or more than 8 weeks (d to r). The dietary level of 18:3n-3 is given. The diets are: corn (b and j); Zephyr (c and 1); peanut (d) (38); lard/corn, 3:1 mixture (e) (14); poppy seed/lard/corn, 4:3:1 mixture (f) (14); olive (g); poppy seed (h) (14); safflower (i) (38); sunflower (k) (14); Tower (m); poppy seed/Tower, 1:1 mixture (n) (14); soybean (o) (38); soybean (p); Tower (q) (14); and linseed (r) (38) oils. Abbreviations: PC = phosphatidylcholine; PE = phosphatidylethanolamine; PUFA = polyunsaturated fatty acids.

from rats fed laboratory chow. Dow-Walsh et al. (42) showed that rats fed diets rich in monoenes and 18:3n-3 had heart mitochondria which aged much faster. It was also found that substantially higher levels of free fatty acids and lyso-PC (21) were produced during the isolation of cardiac lipids particularly from rats fed diets rich in 18:3n-3 using conventional extraction techniques. This suggests that the membranes are more easily dissociated and subject to autolysis before enzymes are inhibited by chloroform/methanol. A "more fragile" membrane could be subject to greater cellular breakdown which could lead to focal necrosis. It is therefore notable that oils containing appreciable amounts of 18:1 (e.g., olive [2,7, 8,20] or peanut oils [43-46]), 18:3n-3 (e.g., soybean [3,4,6,20,46]) or linseed oils [7,8]) or 18:1 and 18:3n-3 (e.g., LEAR oils (2-4,6-10, 14,16,20,33,44-46]) apparently are associated with heart lesions in male rats if fed these oils at a high level in the diet for prolonged periods of time. In fact, both 18:1 and 18:3n-3 were positively correlated with heart lesions in male rats in a statistical analysis carried out on a large number of published results involving cardiopathological examinations in male rats (5).

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