Changes in Liver Lipid Composition of Male Rats Fed Rapeseed Oil Diets[1,2]

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ABSTRACT

Studies are reported on the effect of feeding diets containing rapeseed oils differing in their erucic acid content to male weanling rats for 16 weeks. Rapeseed oil high in erucic acid depressed growth. Total lipids, lipid phosphorous and cholesterol, in the livers were not significantly different between the experimental groups. The fatty acid composition of the total liver lipids, the neutral lipids, phosphatidylethanolamine and phosphatidylcholine are documented. Erucic and eicosenoic acids were found in all lipid classes at the same relative concentration; the amount being incorporated was proportional to that found in the dietary oil. The positional analysis of phosphatidylethanolamine and phosphatidylcholine are presented. Erucic acid was incorporated preferentially at position two of these phospholipids, whereas, twice the level of eicosenoic acid was found at position one, compared to that which occurred at the two position.

INTRODUCTION

Several investigations have been reported on the effect of dietary rapeseed oils on the fatty acid composition of different tissues of the rat (1-10). The comparative effect of rapeseed oils containing high or low levels of erucic (*cis*-13-docosenoic) acid (22:1) was studied (5,7-10). Although data are available for total composition of tissue lipids within the first week of feeding rapeseed oils (6-9) and after long term feeding experiments (10), little is known about the fatty acid composition of particular lipid classes (11,12).

It has been established that feeding rapeseed oil, high in 22:1 concentration, causes severe fat accumulation in heart, adrenal, and skeletal muscle within the first week of feeding. This fatty infiltration gradually disappears after rats are kept several months on these diets (13). The early cardiac fat accumulation is due mainly to an increase in the concentration of triglycerides and free fatty acids, while the phospholipid and cholesterol concentrations remain constant (7, 9,14). The concentration of 22:1 was shown to be much lower in the total phospholipids than in the neutral lipids of the heart (1,9), adrenal (2), and liver (1,3,11,12); in fact no 22:1 was detected in the phospholipids of the liver in long term feeding trials.

In this study male weanling rats were fed for 16 weeks various rapeseed oils which differed in their 22:1 concentration. The livers were analyzed for their total fatty acid composition and for the fatty acid composition of several major lipid classes: neutral lipids, phosphatidylethanolamine (PE), and phosphatidylcholine (PC). In addition the positional distribution of fatty acids in PE and PC was determined.

MATERIALS AND METHODS

Male Sprague-Dawley rats, three weeks old, obtained from Bio-Breeding, Ottawa, Ontario, were selected randomly, caged in pairs, and fed ad libitum for 16 weeks. Five groups of four rats were fed a semisynthetic diet containing 20% casein (vitamin free), 30% cornstarch, 20% sucrose, 1% vitamin mixture (15), 4% USP XIV salt mixture (30 ppm zinc), and 5% alfa floc. Diet one was supplemented with 5% lard and an additional 15% cornstarch; diet two with 20% corn oil; diet three with 20% *Brassica napus* var. Oro (Oro) processed by Cooperative Vegetable Oil Ltd., Altona, Manitoba; diet four with 20% *B. campestris* var. Span (Span) processed by Western Canada Processors, Lethbridge, Alberta; diet five with 20% rapeseed oil, which is obtained from a seed mixture of *B. campestris* var. Echo (15%) and Arlo (85%) (processed by the latter plant). Hereafter, this mixture will be referred to as RSO. After 16 weeks on the diets, the rats were killed by stunning followed by exsanguination. The livers were removed immediately; washed in ice-cold saline, and homogenized in distilled water using a Potter-Elvehjem homogenizer. Total lipids were extracted twice, according to the procedure of Bligh and Dyer (16).

Lipids were transesterified by refluxing with

[1]This article represents part of an extensive experiment carried out by Agriculture Canada to investigate the nutritional value of rapeseed oils (see ref. 15).

[2]Contribution No. 497 from the Animal Research Institute.

TABLE I

Fatty Acid Composition of Dietary Oils[a]

Fatty[b] acid	Mole %				
	Lard	Corn	Oro	Span	RSO
14:0	2.0	trace	0.1	0.1	0.1
16:0	28.4	10.9	5.0	4.0	4.0
16:1	2.8	0.1	0.3	0.3	0.3
18:0	18.0	1.7	2.5	1.7	1.7
18:1	38.1	24.3	62.2	55.9	36.2
18:2	7.9	61.1	18.9	20.0	15.1
18:3	0.4	0.9	6.7	8.3	5.9
20:1	0.8	0.2	1.9	3.9	12.3
20:2	0.4	trace	0.1	0.5	0.5
22:0	—[c]	0.1	0.3	0.4	0.4
22:1	—	—	1.6	4.3	22.3
22:2	—	—	trace	0.3	0.1
24:0	—	0.2	0.3	0.3	1.0
24:1	—	—	—	—	trace

[a]Oro = *Brassica napus* var. Oro; Span = *B. campestris* var. Span; RSO = mixture of *B. campestris* var. Echo and Arlo.

[b]Number of carbon atoms; number of double bonds.

[c]No detectable amounts of fatty acids were observed.

5% (w/w) dry HCl gas in anhydrous methanol for 45 min (17), and the methyl esters purified by thin layer chromatography (TLC). Lipid classes were separated by TLC by means of a two step development procedure (18) to obtain neutral lipids, PE and PC. Bands were visualized under UV light after the chromatograms were sprayed with Rhodamine B.

PE and PC were hydrolyzed enzymatically using snake venom from king cobra (*Ophiophagus hannah*) (Sigma Chemical Co., St. Louis, Mo.) according to Weber et al. (19). The products resulting from phospholipase A hydrolysis were isolated by TLC using $CHCl_3$:CH_3OH:H_2O (65:25:4) as developing solvent.

Free acid methyl esters were analyzed using a gas liquid chromatograph (GLC) (Packard Model 420) equipped with a flame ionization detector. A 200 by 4 mm glass column packed with 5% butanediol succinate on 80/100 mesh Chromosorb G (high performance) was used isothermally at 200 C. Nitrogen was the carrier gas at a rate of 60 ml/min. Peaks were identified by comparison with authentic standards (Nu Chek Prep, Elysian, Minn.) and by the equivalent chain length technique, quantitated by use of a digital integrator (Infotronic 208E), and expressed as mole per cent. Analysis of variance was performed on all data, and significant differences at the 1% level ($P < 0.01$) were determined using Duncan's Multiple Range test. Free and esterified cholesterol (20) and total phosphorous (21) were determined by standard procedures.

RESULTS

The fatty acid composition of the dietary oils is given in Table I. Characteristic low levels of saturated fatty acids were found in all oils; corn oil, however, contained slightly higher levels of palmitic acid (16:0). A high concentration of monoenoic acids, oleic (18:1), eicosenoic (20:1), and erucic (22:1), was common to all rapeseed oils. RSO, Span, and Oro contained decreasing amounts of 20:1 and 22:1, due mostly to a substitution of 18:1. Linoleic acid (18:2) was the major fatty acid in corn oil (57.9%), and it occurred at 15-20% in the rapeseed oils. Linolenic acid (18:3) was found in appreciable amounts (6-8%) in all the rapeseed oils.

Body wt gains of male rats fed diets containing RSO or lard were lower compared to rats fed corn, Oro, or Span oil diets (Table II). Previously it has been reported from this laboratory that a significant increase in hepatic fat occurred in rats fed RSO or Span for two weeks (15). The total lipid, lipid phosphorous, and cholesterol content/g of liver was not significantly different between the treatments

TABLE II

Weight Gain, Liver Weights, Total Liver Lipids, Cholesterol and Phosphorous[a]

Diet	Weight gain g	Liver wet weight g	Total lipids mg/g liver	Total phosphorous mg/g liver	Total cholesterol mg/g liver
Lard	406.7 ± 11.6[b]	15.4 ± 0.7	31.6 ± 3.1	0.60 ± 0.04	1.5 ± 0.3
Corn	443.3 ± 18.1	12.7 ± 1.0	51.0 ± 5.5	0.78 ± 0.05	2.6 ± 0.7
Oro	434.5 ± 18.2	12.1 ± 1.2	37.4 ± 2.2	0.73 ± 0.02	1.8 ± 0.2
Span	433.9 ± 11.7	15.3 ± 1.0	41.6 ± 4.3	0.65 ± 0.05	1.9 ± 0.2
RSO	406.5 ± 6.9	14.3 ± 0.7	38.2 ± 4.9	0.75 ± 0.03	1.4 ± 0.1

[a]Oro = *Brassica napus* var. Oro; Span = *B. campestris* var. Span; RSO = mixture of *B. campestris* var. Echo and Arlo.

[b]Mean ± standard error of the mean of four rats.

TABLE III

Fatty Acid Composition of Total Liver Lipids from Male Rats 16 Weeks on Experimental Diets[a]

Fatty[b] acid	Diet					
	Lard	Corn	Oro	Span	RSO	SEM
14:0	1.1[1f]	0.4[2]	0.3[2]	0.5[2]	0.8[1,2]	0.1
16:0	29.5[1]	25.3[1,2]	19.4[2,3]	15.4[3]	19.3[2,3]	1.3
16:1	7.9	1.0[1]	1.7[1]	2.4[1]	3.2[1]	0.6
18:0	13.2[1,2]	10.6[1,2]	16.3[1]	7.7[2]	11.2[1,2]	1.8
18:1	31.7[2]	16.8	34.2[2]	44.5[1]	35.6[1,2]	2.1
18:2	5.8	31.5	13.9[1]	14.2[1]	11.7[1]	0.8
18:3	trace[2]	0.3[2]	1.6[1]	2.3[1]	1.8[1]	0.2
20:1	0.4[2]	0.6[2]	1.1[1,2]	1.8[1]	3.5	0.2
20:2	0.7[1,2]	0.9[1]	0.3[2]	0.5[2]	0.3[2]	0.08
20:4	6.7[1]	8.4[1]	7.5[1]	7.2[1]	7.2[1]	0.7
22:1	—[g]	—	0.1[1]	0.4[1]	1.7	0.05
22:6	0.8[2]	0.7[2]	1.4[1,2]	0.9[1,2]	1.7[1]	0.2
24:0	0.4[1,2]	0.8[1]	trace[2]	0.1[2]	trace[2]	0.1
Minor S[c]	1.1	1.8	1.3	1.3	1.3	
Minor P[d]	0.7	1.0	0.9	0.7	0.8	
% S	45.3	38.9	37.3	25.0	32.6	
% M	40.0	18.4	37.1	49.1	44.0	
% P	14.7	42.8	25.6	25.8	23.5	
S/U[e]	0.83	0.64	0.59	0.33	0.48	

[a]Oro = *Brassica napus* var. Oro; Span = *B. campestris* var. Span; RSO = mixture of *B. campestris* var. Echo and Arlo.

[b]Fatty acid composition is expressed as molar % of total acids; fatty acids are designated by number of carbon atoms: number of double bonds. S = saturated fatty acids, M = monounsaturated fatty acids, P = polyunsaturated fatty acids, U = unsaturated fatty acids.

[c]Mole % of all trace amounts of saturated fatty acids: 15:0, 17:0 and 20:0.

[d]Mole % of all trace amounts of polyunsaturated fatty acids: 20:3, 20:5, 22:4 and 22:5.

[e]Ratio of total molar % saturated fatty acids to total molar % unsaturated fatty acids.

[f]Analysis of variance was calculated for each set of methyl esters. The mean of four rats on each diet and the pooled standard error of the mean (SEM) are given. Identical superscript numbers indicate no significant difference at the 1% level using Duncan's Multiple Range test.

[g]No detectable amounts of fatty acid were observed.

after the rats were maintained on these diets for 16 weeks (Table II).

The total fatty acid composition of the liver lipids of male rats fed the experimental diets for 16 weeks are presented in Table III. The composition of neutral lipids, PE and PC, isolated from the total hepatic lipids by TLC, are presented in Tables IV, V, and VI, respectively.

Erucic acid was incorporated into the liver lipids of rats maintained on diets containing rapeseed oils: Oro at 0.1%, Span at 0.4%, and RSO at 1.7%. Surprisingly the concentration of 22:1 was found to be the same in both the neutral lipids and phospholipids (PE and PC), contrary to earlier reports that 22:1 was found only in the neutral lipid fraction of the liver lipids (1,3,12). The concentration of 22:1 in the liver lipids was proportional to its concentration in the dietary rapeseed oils but at a much lower concentration.

Although eicosenoic acid was found in the liver lipids of the control groups (lard 0.2-0.4%, corn 0.4-0.6%), much higher concentrations were present in rats fed the rapeseed oils. The concentration of 20:1 appeared to be related to the dietary intake, although the contribution from β-oxidation of 22:1 and chain elongation of 18:1 cannot be eliminated. In rats fed RSO, for example, the 20:1 content in all lipid classes (3.3-3.5%) was greater than the 22:1 content (1.1-1.7%), even though the composition of the dietary RSO showed the opposite relationship (12.3%, 20:1; 22.3%, 22:1). The concentration of 20:1 was identical in the neutral lipids and phospholipids of the liver.

Oleic acid was the major dietary fatty acid in all experimental groups, except those fed corn oil; a corresponding increase in the concentration of 18:1 in all lipid class compositions was observed, in particular that of the neutral lipids. The concentration of 18:1 in the neutral lipid

TABLE IV

Fatty Acid Composition of Neutral Lipids from Male Rat Livers 16 Weeks on Experimental Diets[a]

Fatty acid	Diet					
	Lard	Corn	Oro	Span	RSO	SEM
14:0	1.6	0.5[1]	0.8[1]	0.7[1]	1.0[1]	0.1
16:0	35.2	24.6[1]	19.5[1,2]	13.3[2]	18.5[1,2]	1.4
16:1	9.1	1.0[2]	2.6[1,2]	3.1[1]	3.8[1]	0.4
18:0	2.7[1]	1.9[1]	2.8[1]	1.4[1]	1.6[1]	0.5
18:1	46.0	23.5	56.0[1]	57.8[1]	54.5[1]	1.0
18:2	3.9	42.6	14.3[1]	16.4[1]	11.4	0.5
18:3	trace[2]	0.2[2]	1.5[1]	2.9	2.0[1]	0.2
20:1	0.3[2]	0.4[2]	0.9[1,2]	1.5[1]	3.5	0.2
20:2	0.1[1]	0.5	0.1[1]	0.2[1]	0.2[1]	0.05
20:4	0.4[1]	2.5	0.6[1]	1.0[1]	0.6[1]	0.1
22:1	—	—	0.1[1]	0.3[1]	1.7	0.07
22:6	—	trace[1]	0.1[1]	0.1[1]	trace[1]	0.03
24:0	trace[1]	0.3	—	trace[1]	—	0.02
Minor S	0.7	1.1	0.7	1.1	0.9	
Minor P	—	0.8	0.3	0.1	0.2	
% S	40.2	28.4	23.8	16.5	22.0	
% M	55.2	24.9	59.6	62.7	63.5	
% P	4.4	46.6	16.9	20.7	14.4	
S/U	0.67	0.40	0.31	0.20	0.28	

[a]See Table III Footnotes.

fraction of rats fed lard was 46%, whereas rats maintained on diets containing Oro, Span, or RSO had higher levels of 18:1 which were remarkably similar to each other (Table IV). The latter observation reflected the similarity of the monoenoic acid composition of Oro, Span, and RSO, and was, no doubt, a consequence of rapid β-oxidation of 22:1 and 20:1 to 18:1. The 18:1 level of PC was slightly higher in the groups fed rapeseed oils than in the control groups, whereas in PE the level of 18:1 was increased significantly ($P < 0.01$). A similarity in the level of 18:1 was observed between the three rapeseed oil fed groups,

TABLE V

Fatty Acid Composition of Phosphatidylethanolamine from Male Rat Livers 16 Weeks on Experimental Diets[a]

Fatty acid	Diet					
	Lard	Corn	Oro	Span	RSO	SEM
14:0	0.1[1]	0.1[1]	0.2[1]	0.2[1]	0.3	0.03
16:0	24.7[1]	21.0[1,2]	16.8[2,3]	12.8[3]	17.7[1,2,3]	1.7
16:1	1.5[1]	0.5[2]	0.5[2]	0.8[1,2]	0.8[1,2]	0.2
18:0	32.9[1]	26.2[1]	28.0[1]	23.9[1]	22.8[1]	2.6
18:1	11.1[2]	8.7[2]	16.0[1]	20.0	16.1[1]	0.9
18:2	4.1	14.0[1]	13.7[1]	11.7[1]	9.5[1]	1.0
18:3	—	—	0.3[2]	1.2[1]	0.9[1,2]	0.2
20:1	0.3[1]	0.5[1]	1.0[1]	2.0	3.3	0.2
20:2	0.6[1]	1.2[1]	0.4[1]	0.9[1]	0.8[1]	0.3
20:4	16.9[1]	20.0[1]	15.4[1]	17.7[1]	17.5[1]	2.2
22:1	—	—	0.3[1]	0.5[1]	1.1	0.1
22:6	4.1[1]	2.5[1]	4.7[1]	5.0[1]	6.9[1]	1.1
24:0	1.3[1]	2.5[1]	—	—	—	0.4
Minor S	1.2	1.3	1.3	1.6	1.1	
Minor P	1.2	1.4	1.6	1.6	1.3	
% S	60.2	51.1	46.3	38.5	41.9	
% M	12.9	9.7	17.8	23.3	21.3	
% P	26.9	39.1	36.1	38.1	36.9	
S/U	1.51	1.05	0.86	0.63	0.72	

[a]See Table III Footnotes.

TABLE VI

Fatty Acid Composition of Phosphatidylcholine from Male Rat Livers 16 Weeks on Experimental Diets[a]

Fatty acid	Diet					
	Lard	Corn	Oro	Span	RSO	SEM
14:0	0.3[1]	0.3[1]	0.2[1]	0.2[1]	0.2[1]	0.03
16:0	25.3[1,2]	30.7[1]	21.0[2]	20.2[2]	21.0[2]	1.6
16:1	1.8	0.8[1]	0.2	0.9[1]	0.8[1]	0.1
18:0	33.7[1]	26.1[1]	25.5[1]	24.1[1]	22.6[1]	2.5
18:1	11.5[2]	6.6	13.6[1,2]	16.5[1]	13.4[1,2]	0.7
18:2	6.2[2]	11.6[1]	13.0[1]	9.7[1,2]	9.9[1,2]	0.9
18:3	—	trace	0.6[1]	0.5[1]	0.6[1]	0.2
20:1	0.2[1]	0.5[1]	1.1	2.1	3.5	0.1
20:2	1.4[1]	1.2[1]	0.3	0.7[2]	0.8[2]	0.1
20:4	15.9[1]	18.6[1]	19.3[1]	19.9[1]	20.9[1]	2.6
22:1	—	—	0.2	0.5	1.1	0.05
22:6	1.5[1]	0.9[1]	2.5[1]	2.5[1]	3.3[1]	0.6
24:0	0.7[1]	0.8[1]	0.1[2]	trace[2]	—	0.1
Minor S	0.6	1.3	1.0	1.5	0.9	
Minor P	0.9	0.6	1.6	0.8	1.0	
% S	60.6	59.2	47.8	46.0	44.7	
% M	13.5	7.9	15.1	20.0	18.8	
% P	25.9	32.9	37.3	34.1	36.5	
S/U	1.54	1.45	0.91	0.85	0.81	

[a]See Table III Footnotes.

although the Span group appeared to contain a slightly higher level than either the Oro or RSO fed groups.

The relative concentration of 16:0 was influenced significantly by the different diets, while the concentration of 18:0 was not affected. A marked decrease in the concentration of 16:0 was observed in all lipid classes of all groups fed rapeseed oil compared to the groups fed corn oil or lard. The decrease was most pronounced with the group maintained on Span oil. The level of 18:0 was not significantly different between the experimental groups in either of the neutral lipids, PE or PC.

The concentration of 18:2 in the neutral lipids was related directly to its concentration in the diet. The neutral lipids of the group fed a diet containing lard had the lowest level of 18:2; the group fed the corn oil containing diet had the highest level, whereas the group fed the rapeseed oil(s) containing diets had intermediate levels of 18:2 in the neutral lipids. The influence of dietary 18:2 on the phospholipid composition was much less dramatic. The concentration of 18:2 was significantly lower only in the lard group; differences between the concentration of 18:2 in corn and rapeseed oils did not give rise to significantly different levels of incorporation into the phospholipids (Tables V and VI).

Low levels of 18:3 were found in all lipid classes of rats fed diets containing rapeseed oils. Linolenic acid was incorporated into the neutral lipids (1.5-2.9%) and somewhat less into the phospholipids (0.3-1.2%) of the liver.

The relative proportion of 20:4 in the total lipids of rat livers was not statistically different between the experimental groups. The same pattern was observed in the fatty acid profile of PE and PC isolated from the total liver lipids. On the other hand, the relative concentration of 22:6 appeared to be greater in the phospholipids of rats fed rapeseed oils compared to the control groups. Characteristic differences between PE and PC were maintained; 22:6 was more abundant in PE than in PC.

The ratio of total saturated to total unsaturated fatty acids are included in Tables III to VI. Lower ratios were observed consistently in all liver lipid classes of rats fed diets containing Oro, Span, or RSO, compared to rats fed diets containing lard or corn oil. The ratios in the phospholipids (PE and PC) decreased progressively as the erucic acid content of the dietary rapeseed oils increased. The lower ratios were due to a combination of a lower concentration of saturated and a greater abundance of monounsaturated fatty acids.

The positional distribution of fatty acids esterified to position one and two of PE and PC are presented in Tables VII and VIII, respectively. The characteristic pattern of phospholipids was retained; saturated acids predominated in position one, whereas polyunsaturated

TABLE VII

Distribution of Fatty Acids Esterified to the 1- and 2- Position of Phosphatidylethanolamine from Male Rat Livers 16 Weeks on Experimental Diets[a]

Fatty acid	Diet											
	Lard		Corn		Oro		Span		RSO		SEM	
	1	2	1	2	1	2	1	2	1	2	1	2
14:0	0.6	0.2	0.3	0.3	0.6	0.1	0.2	0.1	0.3	0.2	0.2	0.07
16:0	33.4	14.9	31.8	12.7	25.8	10.7	23.9	6.9	28.4	11.0	1.5	1.4
16:1	1.0	1.3	0.3	0.5	0.4	0.4	—	0.3	0.4	0.6	0.3	0.1
18:0	49.3	21.5	49.1	20.1	54.0	22.8	50.3	15.9	45.5	17.3	2.1	2.7
18:1	11.2	10.7	10.8	7.1	13.2	13.2	17.2	13.5	14.0	12.2	1.5	1.0
18:2	0.3	4.5	1.3	11.3	0.4	7.3	1.0	7.5	1.0	7.0	0.2	1.2
18:3	0.1	—	0.1	trace	—	0.2	trace	0.6	—	0.5	0.03	0.1
20:1	0.5	0.3	0.9	0.4	1.4	1.4	2.5	1.5	5.5	2.9	0.3	0.2
20:2	0.1	1.2	1.6	1.1	0.2	0.5	0.5	0.5	0.5	0.5	0.07	0.08
20:4	0.5	30.6	0.3	32.5	0.3	26.6	0.4	34.9	0.7	28.4	0.1	2.3
22:1	—	—	—	—	—	0.4	—	0.9	0.5	1.6	0.04	0.1
22:6	—	8.7	—	5.2	—	11.7	—	13.8	—	14.6	—	1.7
24:0	—	2.4	0.2	4.1	—	trace	—	0.3	0.1	trace	0.06	0.7
Minor S	3.2	0.8	3.2	2.0	3.5	1.5	4.1	1.0	3.2	1.1		
Minor P	—	2.6	—	2.8	—	3.1	—	2.0	—	2.2		
% S	86.5	39.8	84.6	39.2	83.9	35.1	78.5	24.2	77.5	29.6		
% M	12.7	12.3	12.0	8.0	15.0	15.4	19.7	16.2	20.4	17.3		
% P	1.0	47.6	3.3	52.9	0.9	49.4	1.9	59.3	2.2	53.2		
S/U	6.3	0.66	5.5	0.64	5.3	0.54	3.6	0.32	3.4	0.42		

[a]See Table III Footnotes.

acids predominated in position two. Erucic acid was incorporated preferentially into position two of PE (Oro 0.4%, Span 0.9%, RSO 1.6%) and PC (Oro 0.3%, Span 1.1%, RSO 1.2%). Only trace amounts of 22:1 were found in position one of PE and PC in the groups fed Oro or Span. Ca. one-half as much 22:1 occurred in position two of PE and PC compared to that found in position one of the group fed RSO. Eicosenoic acid, on the other hand, was incorporated preferentially into position one of PE and PC, and occurred in position two at ca. half that concentration. Linolenic acid was incorporated into position one of both phospholipids from rats fed rapeseed oils. Furthermore, the concentration of 22:6 in PE was significantly greater in rats fed rapeseed oils, suggesting a rapid chain elongation and desaturation of 18:3.

The influence of dietary rapeseed oils on the composition of the one and two position in PE was similar; the concentration of saturated fatty acids was lower, of monounsaturated acids was higher, and of polyunsaturated acids not significantly different ($P < 0.01$) from those of the control groups. The greatest changes occurred in rats fed Span and RSO. A similar pattern was observed in the composition of position one in PC. However, the composition of position two in PC was influenced less by the dietary rapeseed oils; the concentration of saturates and mono- and polyunsaturates were between those of the control groups fed corn oil or lard.

The ratio of saturated to unsaturated fatty acids in each position of each diet is given in Tables VII and VIII for PE and PC, respectively. Slightly lower ratios were observed in rats fed rapeseed oils as compared to those fed corn oil or lard. A significant ($P < 0.01$) change in ratio was observed for position one and two of PE and position one of PC, whereas, the ratio in position two of PC remained remarkably similar regardless of the diet fed.

DISCUSSION

Previous investigations have indicated cardiac fat accumulation within the first week of feeding rapeseed oils high in 22:1 to male rats (6-9, 11,13-15) and necrotic heart lesions when these oils were fed for periods of several months (5,7,9,11,13,15,22). Erucic acid has been implicated as causing the lesions (23). Three varieties of rapeseed oils, which differed in their 22:1 content, were studied to determine the extent of incorporation of this acid into the different hepatic lipid classes.

It can be concluded from this study that 22:1 and 20:1 were incorporated ca. equally into hepatic neutral lipids and phospholipids when rapeseed oils containing these acids were fed. This contrasts earlier reports which indi-

TABLE VIII

Distribution of Fatty Acids Esterified to the 1- and 2- Positions of Phosphatidylcholine from Male Rat Livers 16 Weeks on Experimental Diets[a]

Fatty acid	Diet											
	Lard		Corn		Oro		Span		RSO		SEM	
	1	2	1	2	1	2	1	2	1	2	1	2
14:0	0.4	0.2	0.2	0.1	0.2	0.1	0.2	0.1	0.2	0.3	0.04	0.1
16:0	37.0	12.1	36.3	15.9	27.6	11.3	28.3	10.6	28.3	13.3	1.4	1.5
16:1	1.6	2.3	0.6	0.3	0.5	0.5	0.7	0.5	0.5	0.6	0.2	0.2
18:0	44.9	13.1	46.0	15.5	48.3	16.5	47.2	14.5	44.4	15.3	2.9	2.1
18:1	12.0	15.1	7.9	5.4	14.3	13.6	14.1	13.6	12.4	11.1	1.4	1.1
18:2	1.3	11.1	2.2	13.7	1.9	15.7	1.9	13.4	1.8	13.5	0.3	1.1
18:3	—	—	—	—	—	0.3	—	0.4	—	0.2	—	0.1
20:1	0.6	0.3	1.3	0.4	2.9	1.0	3.0	1.2	7.2	2.2	0.6	0.3
20:2	0.2	3.0	2.3	1.2	0.7	0.6	0.7	0.6	0.8	0.6	0.1	0.2
20:4	0.3	31.7	0.3	39.6	0.5	29.7	0.6	34.9	0.8	31.8	0.1	2.9
22:1	—	—	—	—	0.1	0.3	0.1	1.1	0.6	1.2	0.03	0.2
22:6	—	4.8	—	2.7	—	5.8	—	5.9	—	5.6	—	0.6
24:0	—	1.8	—	2.5	—	0.1	—	0.1	—	1.2	—	0.6
Minor S	1.6	0.9	2.8	1.1	2.8	1.2	3.2	1.3	3.2	1.0		
Minor P	—	3.7	—	1.5	—	3.4	—	1.7	—	2.0		
%S	83.9	28.1	85.3	35.1	78.9	29.2	78.9	26.6	76.1	31.1		
%M	14.2	17.7	9.8	6.1	17.8	15.4	17.9	16.4	20.7	15.1		
%P	1.8	54.3	4.8	58.7	3.1	55.5	3.2	56.9	3.4	53.7		
S/U	5.2	0.39	5.8	0.54	3.8	0.41	3.7	0.36	3.2	0.45		

[a]See Table III Footnotes.

cated, after long term feeding trials, that 22:1 was found exclusively in the neutral lipids (1,3,12). The concentration of 22:1 in all lipid classes was in the same ratio as the concentration of this acid in the diet, whereas the concentration of 20:1 showed the proportional relationship, provided an amount present in the control groups is subtracted first. The relative concentration of 22:1 was less than that of 20:1 in all lipid classes, even though in Oro and Span oil both acids occurred at the same relative concentration, and in RSO oil the concentration of 22:1 was twice that of 20:1. The level of 18:1 was significantly higher in the hepatic lipids of rats fed rapeseed oils compared to those fed corn oil or lard, suggesting a rapid β-oxidation of 22:1 and 20:1 to 18:1. Oxidation of long chain monoenoic acids has been demonstrated in rats (24-26). Presumably, 20:1 is a metabolic intermediate in the oxidation of 22:1, accounting for its greater abundance in hepatic lipids (25).

Investigations into the positional distribution of 22:1 and 20:1 in PE and PC revealed that 22:1 was incorporated preferentially into position two and 20:1 into position one. Although no attempts were made to characterize the position of the double bonds in 22:1 and 20:1, it is recognized that erucic acid in rapeseed oils is exclusively 13-*cis*-docosenoic acid and the eicosenoic acid in rapeseed oil a mixture of ca. 75% 11-*cis*- and 25% 13-*cis*-eicosenoic acids (27). Little is known regarding the specific distribution of isomeric long chain monoenes (20:1 and 22:1) in phospholipids. According to Brockerhoff and Ackman (28), 13-*cis*-eicosenoic acid accumulates preferentially in position one and 11-*cis*-eicosenoic acid in either position (variable distribution) of phospholipids. However, they did not determine the distribution of docosenoic acids in rat liver phospholipids (28). In this present investigation, when rapeseed oils were fed to rats, it was observed that 20:1 was present in position one at twice the concentration as that in position two. No attempt was made to distinguish between isomeric 20:1 acids and their origin, be it exogenous or endogenous. An inverse distribution was found for 22:1 in the phospholipids; 22:1 was present in position two at greater abundance than in position one. The pathway by which 22:1 and 20:1 were incorporated into the phospholipids is open to speculation. Hill and Lands (29) have shown that 22:1 did not enter PC via the retailoring route, i.e. acylation of 1-acyl-L-glycerol-3-phosphorylcholine, but that it could be incorporated by acylation of 1-acyl-L-glycerol-3-phosphate, while 20:1 could be incorporated into PC using either substrate.

By expressing the fatty acid composition of the hepatic lipids as a ratio of saturated to

unsaturated fatty acids, a significantly lower ratio was observed for rats fed the rapeseed oil diets in all lipid classes. Generally, the liver lipids of rats fed rapeseed oils had lower concentrations of saturated acids and significantly higher concentrations of monounsaturated acids. A survey of the literature revealed that the monoenoic acid content of PC and PE from normal rat livers is within 6-12%. Representative monoenoic acid concentrations of PC calculated from the data in published papers are: 6.2% (30), 6-8% (31), 7.9 and 9.2% (32), 9.9% (33), 7.2 and 9.8% (34), 7.4% (35), 6.3 and 6.9% (36), 12% (37), 6.5% (38), and 8.2% (39); and of PE are: 7.3-9.1% (31), 6.1 and 9% (34), 8.6% (37), and 5% (38). The monoenoic acid content in PC of rats suffering from essential fatty acid deficiency is within 20 to 30%: 24.7% (33), 24.8% (35), 25.3% (36), 17.7% (39), 24.5% (40), and ca. 20% (41). In the present experiment, rats fed diets containing rapeseed oils had a monoenoic acid concentration between 17 and 23% in PC and PE isolated from the liver lipids. It is tempting to correlate the high concentration of monoenoic acids in PC and PE of rats fed rapeseed oils with a condition similar to that of essential fatty acid deficiency.

Reports have appeared recently in the literature that dermal symptoms similar to essential fatty acid deficiency were observed in hypophysectomized immature rats in spite of an abundance of linoleic and arachidonic acids in the diet, even though only small amounts of 20:3 were observed (42).

It appeared that feeding diets, containing 20% by wt rapeseed oils to rats, brought about a condition of fatty acid imbalance which was not related directly to the concentration of 22:1 and 20:1 found in the hepatic lipid classes. However, a monoenoic acid concentration was found in rats fed rapeseed oils which resembled that found in essential fatty acid deficiency.

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