Changes in the Acyl and Alkenyl Group Composition of Cardiac Phospholipids in Boars Fed Corn Oil or Rapeseed Oil[1]

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ABSTRACT

Boars fed diets containing rapeseed oil for 8 weeks showed significantly higher levels of neutral lipids and similar levels of phospholipids, compared to those fed corn oil. Erucic and eicosenoic acids were found to be high in ethanolamine phosphoglycerides, and in particular alkenyl acyl-ethanolamine phosphoglyceride. Furthermore, both long chain monoenes were incorporated preferentially in position 2 of the choline and ethanolamine phosphoglycerides. The alkenyl group composition of the cardiac lipids of pigs was influenced by dietary fatty acids. When rapeseed oil was fed, small amounts of 20:1 and 22:1 alkenyl constituents were detected.

INTRODUCTION

Several workers have demonstrated that inclusion of rapeseed oil (RSO) high in erucic (*cis*-13-docosenoic) acid in the diets of rats (1-3) and pigs (4-6) resulted in dramatic changes in the fatty acid composition of total cardiac lipids. Changes have also been reported in lipid classes of rat heart (7). In the present communication, a more detailed study is presented of the cardiac polar lipids of pigs fed rapeseed oil from a previous experiment (6) with specific reference to changes in the fatty acid and alkenyl ether composition of the choline and ethanolamine phosphatides.

TABLE I

Relative Concentration of Phospholipids in Cardiac Lipids of Boars Fed Experimental Diets for 8 Weeks

Phospholipid[a]	Diet	
	Corn	RSO[b]
Cardiolipin	8.8 ± 2.2[c]	10.3 ± 1.4
EPG	10.7 ± 2.5	15.9 ± 1.1*
SPG and IPG	5.1 ± 1.6	6.3 ± 0.2
CPG	44.8 ± 1.9	39.3 ± 2.3*
Sphingomyelin	24.3 ± 2.2	24.8 ± 1.5
2-acyl-CPG	6.5 ± 3.1	3.5 ± 1.2

[a]EPG = ethanolamine phosphoglyceride; SPG = serine phosphoglyceride; IPG = inositol phosphoglyceride; CPG = choline phosphoglyceride.

[b]RSO = a seed mixture of *Brassica campestris* var. 'Arlo' (15%) and 'Echo' (85%) containing 12.3% 20:1 and 22.3% 22:1.

[c]Values are mean ± SEM of 3 boars per diet; significant difference between diets at the 5% level (*).

MATERIALS AND METHODS

Yorkshire male pigs (boars), 9 to 10 wk of age, were fed a basl diet supplemented with 20% by weight of either corn oil or rapeseed oil as described previously (6). Three boars from each of the two dietary groups were killed on day zero, and successively after 1, 2, 3, and 8 wk of ad libitum feeding.

Hearts were homogenized and total lipids extracted with $CHCl_3$:MeOH (2:1) according to Christiansen (8). Total lipids were fractionated into neutral and polar lipid fractions by column chromatography on acid-treated Florisil (9). Polar lipids were further fractionated by thin layer chromatography (TLC) using the solvent $CHCl_3$:MeOH:H_2O 65:25:4, and bands were detected under UV light after spraying with a solution of Rhodamine B in methanol. Methyl heptadecanoate was added to all lipid classes to permit quantitation by gas liquid chromatography (GLC).

Transesterification was carried out by reacting the sample for 1 hr at 90 C with anhydrous HCl:MeOH (5% by wt). Methyl esters and dimethyl acetals were separated by TLC using 1,2-dichloroethane as developing solvent (10). Dimethyl acetals were converted to cyclic acetals of 1,3-propanediol (11) before analyses by GLC. Authentic cyclic acetals were prepared from authentic methylwesters (Nu Chek Prep., Elysian, MN); methyl esters were reduced to alcohols with $LiAlH_4$ in anydrous diethyl ether, oxidized to aldehydes with chromium tioxide-pyridine complex in methylene chloride (12), and acetylated with 1,3-propanediol and *p*-toluenesulfonic acid (11) in benzene.

A Hewlett-Packard Model 5830A GLC was used, equipped with flame ionization detectors and a digital integrator. Glass columns (1.8 m x 2 mm) were packed with 5% butanediol succinate on 80/100 mesh Chromosorb G (High Performance) or 10% SP-222-PS (Supelco Inc., Bellefonte, PA). Peaks were identified by co-

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TABLE II

The Fatty Acid Composition of Major Cardiac Lipid Classes of Boars Fed Experimental Diets for 8 Weeks[a]

Fatty acid[b]	TG		CL		CER		EPG		CPG		SPH	
	Corn	RSO	Corn	RSO	Corn	RSO	Corn	RSO	Corn	RSO	Corn	RSO
14:0	0.1[c]	0.1	0.6	0.4	0.9	0.8	0.2	0.5	0.4	0.4	0.7	1.5
16:0 DMA[d]	-	-	-	-	-	-	3.1	2.1	15.6	7.6**	-	-
16:0	19.6	16.3	5.0	6.1	8.1	5.3*	8.0	9.7	13.7	21.6**	16.5	18.5
18:0 DMA	-	-	-	-	-	-	11.0	5.1**	7.5	2.7*	-	-
18:0	21.3	19.4	6.0	10.8	12.1	9.5	32.3	31.2	22.2	19.5	16.9	13.8
18:1	15.1	22.8**	13.6	21.2**	11.7	18.5*	9.5	9.4	12.4	20.4**	5.1	5.8
18:2	35.4	27.0*	66.3	48.1**	55.8	49.8	15.0	8.1**	20.2	15.9*	2.4	1.5*
18:3	0.2	0.6**	0.2	1.5***	0.1	1.1***	0.5	1.1*	0.1	0.5**	-	-
20:0	0.1	0.1	0.2	-	0.2	-	0.1	-	-	-	21.5	20.5
20:1	0.1	4.0***	0.2	5.2***	0.3	5.6***	0.4	9.1***	0.1	3.4***	0.7	1.0
20:2	0.6	0.6	4.4	2.2*	4.8	2.4*	3.4	1.7*	0.9	1.2	-	-
20:4	5.7	4.1	0.4	0.7	3.3	0.7	13.3	7.2*	4.5	3.9	-	-
22:0	-	-	0.2	0.2	0.1	-	-	-	-	-	12.1	10.1
22:1	-	2.7	0.2	1.4***	0.2	1.8***	-	11.1	-	2.5	-	2.0
23:0	-	-	-	-	-	-	-	-	-	-	3.5	1.0*
24:0	-	-	-	-	-	-	-	-	-	-	10.7	11.8
24:1	-	-	-	-	-	-	-	-	-	-	4.5	9.0**

[a]TG = triglyceride; CL = cardiolipin; CER = cerebrosides; EPG = ethanolamine phosphoglyceride; CPG = choline phosphoglyceride; SPH = sphingomyelin.

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

[c]Each value represents the mean of three animals per diet. Significant difference between diets at 5 (*), 1 (**) and 0.1% (***).

[d]DMA = dimethyl acetal.

TABLE III

Aliphatic Moieties in the 1 and 2 Position of Ethanolamine Phosphoglycerides (EPG) and Choline Phosphoglycerides (CPG) from Cardiac Lipids of Boars[a]

Chain length: number of double bonds	1-Acyl				2-Acyl			
	EPG		CPG		EPG		CPG	
	Corn	RSO	Corn	RSO	Corn	RSO	Corn	RSO
16:0	8.7	13.2	41.6	24.7	13.2	9.6	30.2	31.9
18:0	78.0	61.2*	47.2	47.2	42.2	33.6*	31.4	24.1
18:1	2.5	6.2*	1.2	5.6***	14.3	15.0	16.3	23.2
18:2	0.9	1.8	0.9	1.5	14.3	10.1	17.5	11.3
18:3	0.5	0.5	0.1	1.2*	0.1	0.1	-	0.2
20:1	0.1	2.1***	0.1	2.8***	0.8	12.4***	0.2	4.5***
20:2	0.2	0.1	0.1	0.3	2.0	1.9	0.7	0.8
20:4	0.4	0.5	0.4	0.1	7.3	5.3	1.7	1.2
22:1	-	4.4	-	5.4	-	8.7	-	2.7
22:4	-	-	-	-	1.0	0.3**	0.4	0.1
	1-Alkenyl ether				2-Acyl			
16:0	23.6	17.4*	55.1	38.3**	21.3	21.1	3.6	3.3
17:0	2.1	3.8	2.3	2.8	trace	trace	trace	trace
18:0	44.8	29.7**	24.0	22.2	20.1	13.4	1.7	2.7
18:1	20.2	27.1**	14.0	25.9**	9.3	9.9	16.1	23.0**
18:2	5.5	1.1**	4.2	1.1**	18.3	11.4	54.5	46.2*
18:3	-	-	-	-	-	0.3	-	2.1
20:0	1.4	1.6	0.4	0.6	trace	trace	trace	trace
20:1	0.2	2.4***	0.1	2.1***	0.7	9.3***	0.3	3.3***
20:2	0.7	2.2*	0.2	0.5	2.9	3.1	2.2	2.2
20:4	-	-	-	-	17.9	7.4**	17.1	11.3*
22:1	-	3.5	-	1.7	-	18.6	-	2.1
22:4	-	-	-	-	3.5	1.6*	1.9	0.6**

[a]Three boars per diet. Each value represents the mean of three animals. Significant differences between diets are indicated at the 5% (*), 1% (**), and 0.1% (***) level.

[b]Traces of fatty acids not included in both phosphatides: 14:0, 15:0, 17:0, 20:0, 20:3, 20:5, 22:5n-6, 22:5n-3, and 22:6. Traces of alkenyl ethers not included: 12:0, 14:0, 15:0, and 22:0.

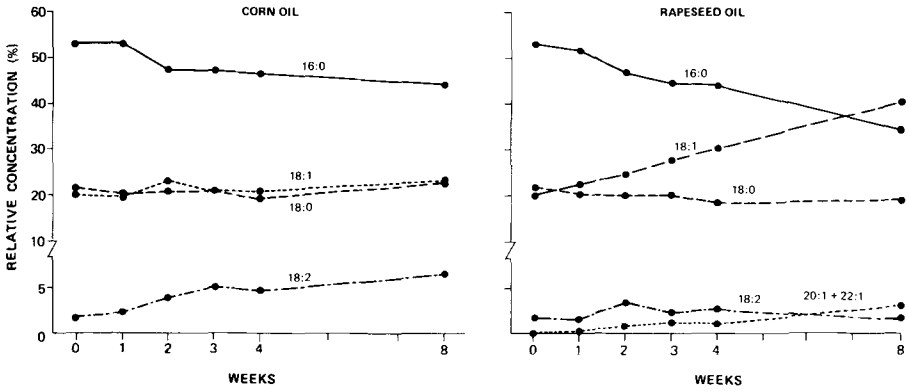

FIG. 1. The relative concentration of the major alkenyl ethers in the total cardiac lipids of boars fed diets containing corn oil or rapeseed oil for 8 wk.

chromatography with authentic standards, by hydrogenation and subsequent GLC analysis, and by prior separation by argentation-TLC (13) followed by GLC analysis.

The alkenyl ethers of the choline and ethanolamine phosphoglyceride were hydrolyzed by exposure to fumes of HCl (14) and the reaction products and unreacted phosphoglycerides were isolated by TLC using the solvent system $CHCl_3$:MeOH:H_2O 65:25:4. The relative concentration of alkenyl-acyls to diacyls was determined by the addition of methyl heptadecanoate as internal standard to the 2-acyl and diacyl phosphoglyceride. The diacyl phosphoglycerides were hydrolyzed enzymatically according to Weber et al. (15) to permit positional analysis. The relative abundance of alkyl-acyl to diacyl phosphoglycerides was obtained by reacting the unreacted phosphoglycerides above with $LiAlH_4$ and estimating the reaction products by TLC (16).

RESULTS AND DISCUSSION

As shown previously (6), heart weights and total cardiac lipids of boars fed diets containing either corn oil or RSO for 8 wk were not significantly different. Results from this study indicate that the cardiac phospholipids were not significantly affected (mg P/g of wet tissue: corn 0.36 ± 0.06, RSO 0.30 ± 0.03), but neutral lipids were significantly higher ($P<0.05$) when RSO was fed (% lipid: corn 30 ± 3, RSO 40 ± 6). A slight elevation of neutral lipids in hearts of pigs fed rapeseed oils has been observed previously (17,18).

The relative concentration of most polar lipids was remarkably similar in the two diets (Table I). Small differences ($P<0.05$) were observed between the relative abundance of choline and ethanolamine phosphoglycerides. In contrast, studies with rats indicate a decrease ($P<0.05$) of glycerophosphoryl ethanolamine (GPE) and an increase ($P<0.01$) of sphingomyelin in cardiac phospholipids (19).

The fatty acid composition of the major lipid classes is shown in Table II. When the diet containing rapeseed oil was fed, the relative concentrations of 18:1, 18:3, 20:1, and 22:1 increased in the total cardiac lipids (6). As seen in Table II, all subclasses showed the same trends, higher levels of these acids in pigs fed RSO compared to pigs fed corn oil. The highest concentration of long chain monoenes (20:1 and 22:1) was found in the ethanolamine phosphoglycerides. This is in marked contrast to the findings in rat heart lipids where a high concentration of 20:1 and 22:1 was found in triglycerides (2) and cardiolipin (7).

The relative abundance of the alkenyl-acyls to diacyls was not significantly affected by diet in either the ethanolamine (% of phospholipid: corn 40 ± 10%, RSO 42 ± 6%) or choline: (corn 45 ± 8%, RSO 39 ± 6%) phosphoglycerides. The amount of alkyl-acyls relative to diacyls in either phosphoglyceride was judged by TLC to be less than 5% from the presence of 1-alkyl glycerol after reduction of the unreacted phosphoglyceride obtained by acid hydrolysis with $LiAlH_4$. The contribution of the fatty acid of alkyl-acyl phosphoglycerides to the analysis of position 2 of the diacyl phosphoglyceride was therefore considered to be negligible.

The positional analysis of diacyl- and alkenyl acyl-choline phosphoryl ethanolamine (CPE) and -glycerophosphoryl choline (GPC) is shown in Table III. Characteristic differences were observed as expected between positions 1 and 2 and between the two phosphoglycerides. For example, the 1-position of the diacyl phospho-

glycerides was mainly saturated; 1-acyl-GPE contained predominantly 18:0, while 1-acyl-GPC contained equal amounts of 16:0 and 18:0. Position 2 of diacyl phosphoglycerides contained higher levels of unsaturated fatty acids compared to position 1. Differences were also apparent between position 2 of the alkenyl derivatives of both ethanolamine and choline phosphoglycerides which contained relatively more linoleate and arachidonate than the corresponding diacyl derivatives. The alkenyl group composition consisted mainly of 16:0, 18:0, and 18:1 moieties identified as aldehydes after acid hydrolyses. The 1-alkenyl-GPE was rich in the 18:0 moiety and 1-alkenyl-GPC contained high levels of the 16:0 derivative.

Feeding the diet containing rapeseed oil to pigs resulted in marked changes in the fatty acid composition of the ethanolamine and choline phosphoglycerides from pig heart (Table II). Significant amounts of long chain monoene fatty acids 20:1 and 22:1 were found in all acyl positions. Generally, the relative concentrations of 20:1 and 22:1 fatty acids were higher in position 2 compared to position 1 in both phosphatides; similar levels were found in position 1 between the two phosphatides; and higher levels were detected in 2-acyl-GPE than in 2-acyl-GPC. In an earlier study with liver phospholipids of rats fed the same rapeseed oil, 22:1 was also incorporated preferentially in position 2 of ethanolamine phosphoglyceride and choline phosphoglyceride, but 20:1 was found to be higher in position 1 of these phosphoglycerides (20).

The highest level of 22:1 was found in the 2-acyl position of alkenyl acyl-GPE. The accumulation of this acid could be due first to a slower enzymatic hydrolysis of erucyl esters compared to esters of common fatty acids (3). Indeed, this hypothesis has been used to explain the high levels of cholesteryl erucate in the adrenals of rats fed rapeseed oil (21). Secondly, acyltransferase activity of 1-alkenyl-phosphoglycerides was demonstrated to be low in many tissues examined (22,23) and may be further reduced by stress such as in the case of essential fatty acid deficiency (24). The lower enzymatic hydrolysis of erucate esters, and the possible stress on animals fed high fat diets (25), in particular, diets containing rapeseed oils high in 22:1 (26), could lead to an accumulation of this acid in alkenyl acyl-GPE.

As seen in Figure 1, the alkenyl group composition was influenced by the kind of fatty acids in the diet. Feeding a diet containing rapeseed oil resulted in a marked increase in the level of the 18:1 alkenyl ether moiety, and in the presence of 20:1 and 22:1 alkenyl ether groups. Analysis of the constituent alkenyl ethers of the two phosphoglycerides indicated a similarity in the level of 18:1, 20:1, and 22:1 alkenyl ethers. Although the composition of the major alkenyl constituents 16:0, 18:0, and 18:1 was strikingly different from that of the fatty acids on position 1 of the corresponding diacyl phosphoglycerides, a similarity in the level of 20:1 and 22:1 moiety was observed. These results clearly demonstrate that the erucate and eicosenoic esters can serve as precursers of alkenyl ethers.

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REFERENCES

1. Walker, B.L., Nutr. Metabol. 14:8 (1972).
2. Rocquelin, G., J.-P. Sergiel, P.O. Astorg, and R. Cluzan, Ann. Biol. Anim. Bioch. Biophys. 13:587 (1973).
3. Kramer, J.K.G., S. Mahadevan, J.R. Hunt, F.D. Sauer, A.H. Corner, and K.M. Charlton, J. Nutr. 103:1696 (1973).
4. Walker, B.L., Can. J. Anim. Sci. 52:713 (1972).
5. Molnar, S., U. ter Muelen, and H. Rosenow, Z. Tierphysiol., Tierernährg. u. Futtermittelkde 29:196 (1972).
6. Kramer, J.K.G., D.W. Friend, and H.W. Hulan, Nutr. Metabol. 19:279 (1975).
7. Blomstrand, R., and L. Svensson, Lipids 9:771 (1974).
8. Christiansen, K., Anal. Biochem. 66:93 (1975).
9. Carroll, K.K., JAOCS 40:413 (1963).
10. Winterfeld, M., and H. Debuch, Hoppe-Seyler's Z. Physiol. Chem. 345:11 (1966).
11. Venkata, Rao, P., S. Ramachandran, and D.G. Cornwell, J. Lipid Res. 8:380 (1967).
12. Ratcliffe, R., and R. Rodehorst, J. Org. Chem. 35:4000 (1970).
13. Wood, R., and F. Snyder, JAOCS 43:53 (1966).
14. Schmid, H.H.O., and H.K. Mangold, Biochim. Biophys. Acta 125:182 (1966).
15. Weber, E.J., I.A. de la Roche, and D.E. Alexander, Lipids 6:525 (1971).
16. Wood, R., and F. Snyder, Ibid. 3:129 (1968).
17. Friend, D.W., A.H. Corner, J.K.G. Kramer, K.M. Charlton, F. Gilka, and F.D. Sauer, Can. J. Anim. Sci. 55:49 (1975).
18. Vodovar, N., F. Desnoyers, R. Levillain, and R. Cluzan, C.R. Acad. Sci. Paris 275D:1597 (1973).
19. Beare-Rogers, J.L., in "Modification of Lipid Metabolism," Edited by E.G. Perkins and L.A. Witting, Academic Press, Inc., New York, NY, 1975, p. 43.
20. Kramer, J.K.G., Lipids 8:641 (1973).
21. Carroll, K.K., Can. J. Biochem. Physiol. 40:1115 (1962).
22. Lands. W.E.M., and P. Hart, Biochim. Biophys. Acta 98:532 (1965).
23. Waku, K., and W.E.M. Lands, J. Biol. Chem. 243:2654 (1968).
24. Blank, M.L., R.L. Wykle, and F. Snyder, Biochim. Biophys. Acta 316:28 (1973).

25. Hulan, H.W., J.K.G. Kramer, and A.H. Corner, Can. J. Physiol. Pharmacol. (In press).
26. Hulan, H.W., W.G. Hunsaker, J.K.G. Kramer, and S. Mahadevan, Ibid. 54:1 (1976).

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