Black Currant Seed Oil Feeding and Fatty Acids in Liver Lipid Classes of Guinea Pigs

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Guinea pigs were fed one of three diets containing 10% black currant seed oil (a source of gamma-linolenic (18:3 n-6) and stearidonic (18:4 n-3) acids), walnut oil or lard for 40 days. The fatty acid composition of liver triglycerides, free fatty acids, cholesteryl esters, phosphatidylinositol, phosphatidylserine, cardiolipin, phosphatidylcholine and phosphatidylethanolamine were determined.

Dietary n-3 fatty acids found esterified in liver lipids had been desaturated and elongated to longer chain analogues, notably docosapentaenoic acid (22:5 n-3) and docosahexaenoic acid (22:6 n-3). When the diet contained low amounts of n-6 fatty acids, proportionately more of the n-3 fatty acids were transformed. Significantly more eicosapentaenoic acid (EPA) (20:5 n-3) was incorporated into triglycerides, cholesteryl esters, phosphatidylcholine and phosphatidylethanolamine of the black currant seed oil group compared with the walnut oil group.

Feeding black currant seed oil resulted in significant increases of dihomogamma-linolenic acid (20:3 n-6) in all liver lipid classes examined, whereas the levels of arachidonic acid (20:4 n-6) remained relatively stable. The ratio dihomo-gamma-linolenic acid/arachidonic acid was significantly (2.5-fold in PI to 17-fold in cholesteryl esters) higher in all lipid classes from the black currant seed oil fed group.
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The enrichment of tissue lipid classes with the 20-carbon chain length fatty acids that are precursors of the various eicosanoids is basic to the idea that dietary fats can affect eicosanoid synthesis and those biological functions they mediate. The eicosanoids derived from these different fatty acids can have differing and even opposing effects. For example, eicosanoids of the 1 series derived from dihomo-gamma-linolenic acid (DHLA; 20:3 n-6) have antiaggregatory effects on platelets (1), whereas eicosanoids of the 2 series derived from arachidonic acid (AA; 20:4 n-6) are proaggregatory (2, 3). Thus, tissue fatty acids favorably modulated by dietary fat intake might lead to synthesis of a complement of eicosanoids having a positive influence on health. Dietary supplementation with DHLA has been shown to result in an increased PGE^1/PGE^2 ratio production by human platelets (4) and a decreased aggregatory response (4, 5).

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Abbreviations: AA, arachidonic acid; BCO, black currant seed oil; CE, cholesteryl esters; CL, cardiolipin; DHA, docosahexaenoic acid; D HLA, dihomo-gamma-linolenic acid; EPA, eicosapentaenoic acid; FFA, free fatty acid; LA, linoleic acid; LN, alpha-linolenic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SA, stearidonic acid; TG, triglyceride; WO, walnut oil; HPTLC, high performance thin layer chromatography.

Black currant seed oil (BCO) is an excellent source (15-19%) of gamma-linolenic acid (GLA; 18:3 n-6), the Δ6-desaturase product of linoleic acid (LA; 18:2 n-6). Because the activity of this enzyme can be compromised under certain conditions by hydrocortisone, adrenalin, glucagon (6), aging (7, 8), cirrhosis (9, 10), diabetes (11) and malnutrition (10, 12), it is hypothesized that certain of the undesirable effects of these conditions could be due to a relative deficit of GLA, DHLA, AA, or their cyclooxygenase metabolites. BCO also contains 12-14% alpha-linolenic acid (LN; 18:3 n-3), a nutritionally essential fatty acid, and 2-4% stearidonic acid (SA; 18:4 n-3), its Δ6-desaturase product. The elongation product of SA, by virtue of its competition for the Δ5 desaturase, may be important not only by inhibiting the transformation of GLA to AA but also because it can be elongated and desaturated to eicosapentaenoic acid (EPA, 20:5 n-3), the precursor of eicosanoids of the 3 series. BCO is thus an interesting oil in several respects.

In the rat, feeding sources of GLA results in increased incorporation of GLA in triglycerides (TG) (13) or DHLA in total lipids (14) but no or slight incorporation into the phospholipids. Phospholipids of the mouse (15-16) are similarly intractable to the influence of dietary GLA. Lipids and phospholipids of humans are more amenable to dietary modulation of n-6 fatty acids. With a single 1 g oral dose, DHLA is incorporated into blood triglycerides within a few hours and into phosphatidylcholine (PC) within 24 hr (17). Sustained oral dosing results in incorporation of DHLA into the membrane of red blood cells and into all lipid classes (17). DHLA levels in serum phospholipids and cholesteryl esters are also sensitive to LA intake (18).

Stone et al. (17) compared the Δ5-desaturase activity from different species and found that in the rat and mouse it is especially active, whereas liver homogenates from man, rabbit (17) or guinea pig (17, 19) show only slight ability to desaturate DHLA to AA. Therefore, in the present experiment, we considered that the guinea pig would be preferred over the rat as an animal model which better reflects the situation in man. The liver being the major site of fatty acid conversions was chosen as the tissue in which to study the effect of diet on fatty acid composition.

The formation of eicosanoids requires the mobilization of substrate as fatty acid, preferentially in a nonesterified form (20, 21). If this is true, there must be *a priori* hydrolysis of esterified lipid classes before prostanoid synthesis can take place (20, 22). Phospholipids are major contributors of fatty acids for prostaglandin synthesis (23) and the consensus is that substrate supply is a limiting factor (24-26). The fatty acid content of triglycerides reflects to a large extent the dietary fat but may also contribute to those pools of free fatty acids susceptible to oxygenation.

The aim of this study was to investigate whether guinea pig liver lipids could be enriched in DHLA by

feeding BCO and if some of these lipid classes were more sensitive than others.

MATERIALS AND METHODS

Animals and diets: Male Dunkin-Hartley guinea pigs weighing ca. 300 g (KFM, Fullinsdorf, Switzerland) were randomly allocated into 3 groups of 7 animals and maintained in individual Macrolon (type III) cages. Each group was fed a nutritionally adequate, semisynthetic diet containing BCO (F.I.S., Chatel St. Denis, Switzerland), walnut oil (WO) (Du Baron, France), or lard (Table 1) *ad libitum* for 40 days. BCO was stabilized with ascorbyl palmitate (200 ppm). The diets were refrigerated and fed fresh each day to the animals. Any leftover diet was discarded. The fatty acid compositions of the diets are shown in Table 2. Walnut oil was chosen as a control for BCO because it is lacking in GLA and SA, but compensates with more LA such that the total quantity of n-6 and n-3 fatty acids, as well as their ratios, are similar in the two oils. All animals had free access to water which contained 250 mg/liter of ascorbic acid. They were weighed weekly.

After an overnight fast, animals were anaesthetized with pentobarbital. Livers were quickly excised and freeze clamped with tongs which had been cooled in liquid nitrogen. Frozen tissue was ground to a fine powder with dry ice and conserved at −80°C until analysis.

Analytical methods. Sample extraction: A 1-g sample of frozen liver tissue was homogenized in 20 vol of chloroform/methanol (2:1, v/v) and total lipids were extracted according to the method of Christiansen (27).

Separation of lipid fractions: Neutral lipids were separated from polar lipids on SepPak cartridges, (Waters Associates, Framingham, MA) (28). Nonacidic and acidic polar lipid fractions were separated on ion-exchange DEAE-Sephadex A-25 (Pharmacia, Sweden) disposable minicolumns (29, 30). The acidic polar lipid fraction was then desalted using a minicolumn of Sephadex G-25 (Pharmacia, Sweden) (31).

Separation of lipid classes: Separation of the lipid classes was performed in duplicate. Neutral lipids were separated by linear high performance thin layer chromatography (HPTLC) on washed silicic acid plates (Merck, ref. 60F254). The plates were developed with petroleum benzine/diethyl ether/acetic acid (85:15:0.5, v/v/v), air-dried, sprayed with 0.005% primuline (w/v) in acetone; the lipid bands were visualized under ultraviolet light. Cholesteryl esters (CE), triglycerides and free fatty acids (FFA), identified by comparison with standards, were scraped off and collected into glass tubes sealed with teflon lined caps.

Polar lipid extracts were separated by linear chromatography (HPTLC) on silicic acid plates (Merck, ref. 60F254) impregnated with 2% boric acid in absolute methanol (w/v) and developed with chloroform/methanol/triethylamine/water (30:25:34:8, v/v/v/v) (29). The acidic phospholipids (phosphatidylserine [PS], phosphatidylinositol [PI], cardiolipin [CL] and the nonacid phospholipids: phosphatidylethanolamine [PE], and phosphatidylcholine [PC]) were visualized as for the neutral lipids, identified via standards, and collected as described above.

Transesterification: All transesterifications were carried out in the presence of silica gel plate materials. For triglycerides, the method was adapted from Shehata et al. (32). Triglycerides were extracted from the silica powder with 1 ml diethyl ether/hexane (2:1, v/v) and 1 ml of 2 M methanolic sodium methoxide was added. The mixture was ultrasonicated for 15 min at room temperature and subsequently washed with 1 ml distilled water.

Cholesteryl esters were extracted with 1.5 ml of diethyl ether/hexane (2:1, v/v) and transesterified with 0.5 ml of the above reaction mixture for 20 min at 50°C. Fatty acid methyl esters were taken up in hexane. We found no selectivity for the various fatty acids in the transesterification process under the given conditions as has been proposed by Epps and Kaluzny (33).

TABLE 1

Composition of the Basic Diet Fed to the Experimental Guinea Pigs

Ingredient	g/100 g
Casein	30.0
Starch	20.0
Sucrose	10.0
Glucose	3.8
Cellulose	15.0
Mineral mix[a]	6.0
Vitamins[b]	2.2
Potassium acetate	2.5
Magnesium oxide	0.5
Fat[c]	10.0

[a]As per M.R.S. Fox and G.M. Briggs (1960) *J. Nutr. 72*, 243-250.
[b]Vitamin fortification mixture, I.C.N. Biochemicals, Cleveland, OH.
[c]Black currant seed oil, walnut oil or lard.

TABLE 2

Fatty Acid Composition of the Guinea Pig Diets (Molar %)

Fatty acid[a]	BCO[b]	WO[b]	Lard
14:0	—	—	2.7
16:0	7.4	8.6	32.4
16:1	—	—	3.4
17:0	—	—	0.5
17:1	—	—	0.3
18:0	0.8	1.9	14.2
18:1	10.4	20.8	36.5
18:2 n-6	48.1	57.7	8.4
18:3 n-6	17.1	—	—
18:3 n-3	12.7	11.0	0.7
18:4 n-3	2.6	—	—
20:1 n-9	0.6	—	0.7
20:2 n-6	0.3	—	—
n-6 sum	65.5	57.7	8.4
n-3 sum	15.3	11.0	0.7
n-6/n-3 ratio	4.3	5.2	12.0
Unsat index[c]	207.6	169.2	59.8

[a]Fatty acids are designated by the number of carbon atoms followed by the number of double bonds. n indicates the position of the first double bond relative to the methyl end of the molecule.
[b]BCO; black currant seed oil. WO; walnut oil.
[c]Unsaturation index: sum of (a × b); a is the relative molar % of each unsaturated fatty acid, b is the number of double bonds for that particular fatty acid.

Free fatty acids were extracted with 1 ml of diethyl ether/hexane (2:1, v/v) and esterified with 1 ml of 2% sulfuric acid in methanol for 20 min at 80°C (34). The fatty acid methyl esters were then neutralized with potassium carbonate.

Polar lipids were transesterified in the same manner as were TG, except that they were extracted with methanol.

Fatty acid methyl esters from all lipid classes were extracted into hexane (Baker resianalyzed) for gas chromatographic analysis.

Gas chromatography: All gas chromatographic analyses were performed on a CARLO-ERBA model 5160 Mega series gas chromatograph (Milan, Italy) equipped with an automatic cold on-column injector, a tailor-made capillary column, coated with immobilized polyethylene glycol (35) and a flame ionization detector. The gas chromatographic conditions were: a fused silica column of 27 m × 0.32 mm I.D. coated with Carbowax 20M; a fused silica precolumn of 1 m × 0.53 mm I.D. The hydrogen inlet pressure was 60 kPa. The oven temperature program was: 80°C, 2 min isothermal, 15°C/min to 140°C, 1 min isothermal, 4°C/min to 220°C (36). The detector was set at 320°C. Chromatograms were recorded using a Spectra Physic 4270 integrator (San Jose, CA). Identification of peaks was made by comparison of retention times with those of a standard (NuChek Prep, Elysian, MN) run under the same conditions.

Statistics: Effect of diet was submitted to analysis of variance and comparison between diets was made using Newman-Keuls studentized range test.

Comparisons between liver fatty acids for a given diet were performed using paired t-tests. Values of $p<0.05$ were considered statistically significant.

RESULTS

Among the three groups of animals, neither the final body weights (BCO = 479 ± 35 g; WO = 454 ± 55 g; and lard = 445 ± 50 g), nor the total liver weights (BCO = 21.6 ± 1.5 g; WO = 18.6 ± 4.1 g; and lard = 17.5 ± 4.3 g) were different.

n-6 Fatty acids. Of the n-6 fatty acids (Figs. 1-3), LA incorporated in the greatest amounts in all lipid classes except PE where AA was also incorporated to a large extent.

The content of LA in the liver TG from guinea pigs

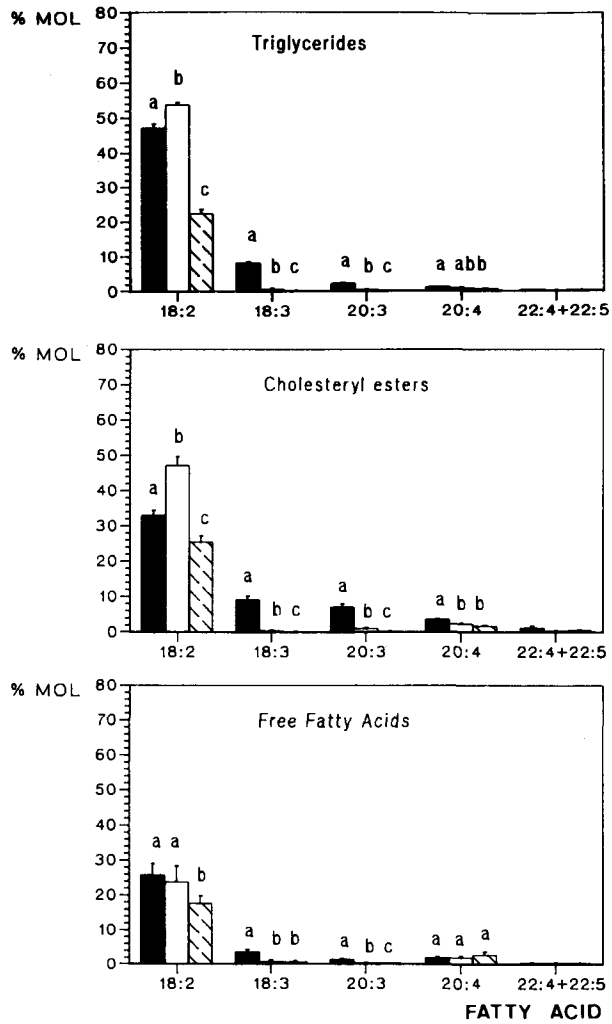

FIG. 1. n-6 fatty acids (mol %) in triglycerides (TG), cholesteryl esters (CE) or free fatty acids (FFA) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7-8 animals ± SEM) with different letters are significantly different.

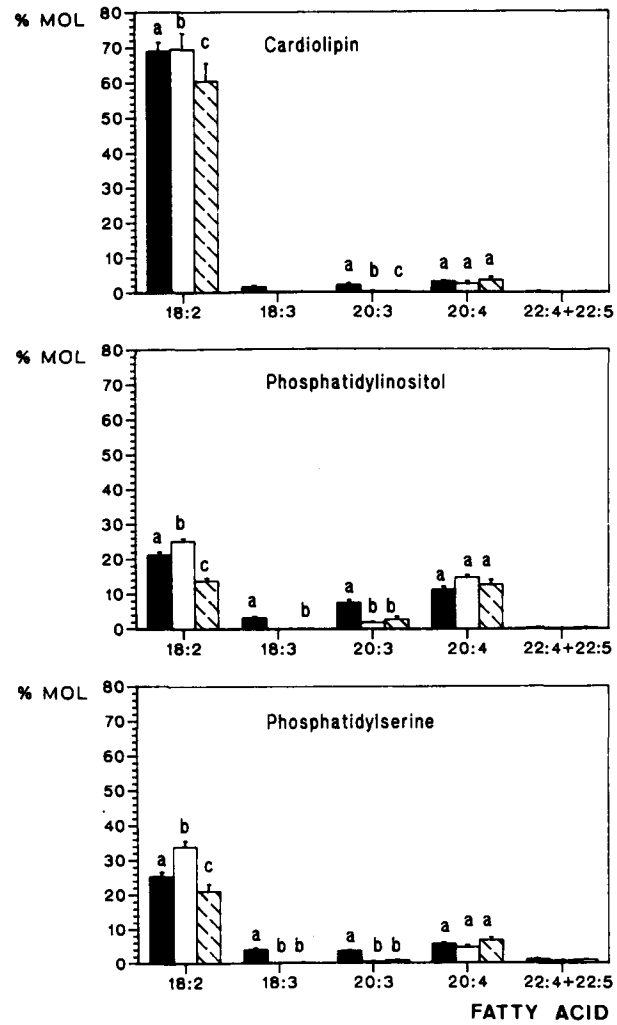

FIG. 2. n-6 fatty acids (mol %) in cardiolipin (CL), phosphatidylinositol (PI) or phosphatidylserine (PS) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7-8 animals ± SEM) with different letters are significantly different.

eating the BCO and WO diets was proportionally similar to the content (Table 2) of the diets. In the TG of the lard-fed group, LA proportion exceeded by about 2.5-fold that of the diet. In most of the other lipid classes except for CL and FFA, the levels of LA were roughly related to the dietary proportion: greater in liver of animals eating the WO diet compared with the BCO fed group, and least in the lard-fed group. As with the TG, lipids from the lard group contained more LA than would be expected from the proportion in the diet. In most lipid classes, LA was found at levels of 13–33%, the exception being in CL levels which were much higher (60–70%), even in livers from those animals which ate the lard diet.

The content of both GLA and DHLA was also affected by dietary fat, being greater in liver lipids from BCO fed animals compared with the WO- and lard-fed animals. This was true for all lipid classes. In the BCO group, more GLA (available from the diet) than DHLA was found in TG, FFA and PC. In the other phospholipids, the content of these two fatty acids was similar except in PI and in CL, where DHLA incorporation exceeded that of GLA.

AA, although varying in quantity from lipid class to lipid class, did not show the large differences between dietary groups noted for other fatty acids. This acid was only slightly elevated in PC, PE and CE by BCO feeding.

The shifts upward of DHLA and no change or slight upward shift of AA resulted in ratios of DHLA/AA (Fig. 4) being significantly increased in the group of guinea pigs fed BCO compared with the other groups.

The longer chain n-6 fatty acids, 22:4 and 22:5, were found in very small amounts and, while differing between lipid classes, did not significantly vary with respect to dietary treatment.

n-3 Fatty acids. The n-3 fatty acid data are shown in Figures 5–7. LN was found to a much lesser extent in the liver lipids of the guinea pig than had been LA.

Where the diet contained substantial quantities of LN (BCO and WO), its content was greater than when the diet contained little LN (lard). In the livers of animals fed the lard diet, there was less conservation of LN as there had been for LA. The levels of LN in the neutral lipids (TG, FFA and CE) were the greatest, indicating that these lipid classes have the most direct relation to dietary intake. In the phospholipids, however, the level of this acid was low (0.1–5.4%); of these, CL incorporated the most, as it did of LA.

In the phospholipids of animals fed the lard diet, and in PE and PS from the animals fed the other diets, the content of the longer chain n-3 fatty acids exceeded that of LN, with docosahexaenoic acid (DHA; 22:6 n-3) being the fatty acid found to the greatest extent. EPA content was significantly greater in TG, CE, PC and PE of the BCO fed group compared with the WO fed animal. Significantly, more EPA was also found in the TG and CE of the BCO group than of the lard group.

Unsaturation index. The liver unsaturation index, or the sum of the molar % of each fatty acid multiplied by its number of double bonds (Fig. 8), did not reflect the gross differences in saturation found in the dietary fatty acids (Table 2). This similarity was not found in neutral lipids, and a lesser unsaturation index was also noted in CL and PC in the animals eating the highly saturated lard diet.

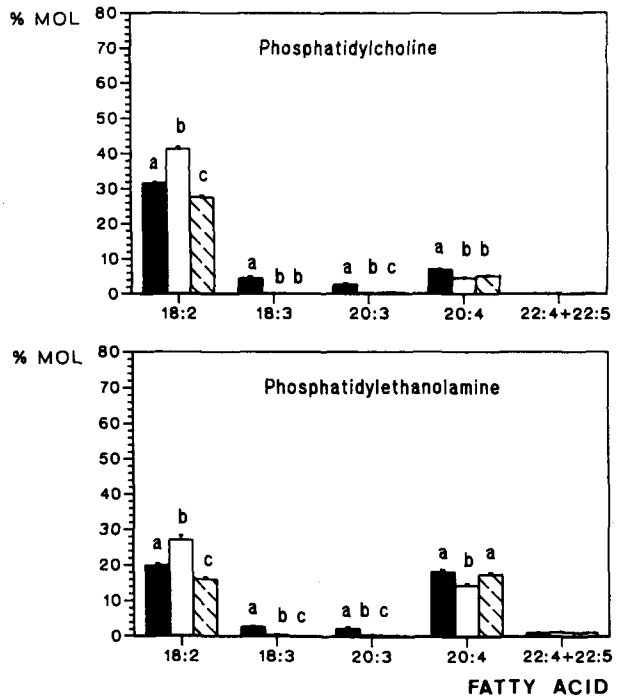

FIG. 3. n-6 fatty acids (mol %) in phosphatidylcholine (PC) or phosphatidylethanolamine (PE) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7–8 animals ± SEM) with different letters are significantly different.

DISCUSSION

The observation that LA incorporation into liver lipids was in proportion to the dietary content is consistent with results obtained in human plasma (18). In the lard-fed group, incorporation of LA into liver was dis-

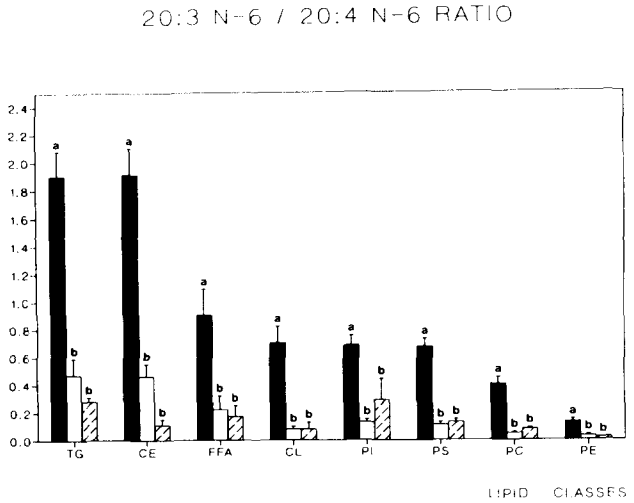

FIG. 4. The ratio of molar % dihomo-gamma-linolenic acid over molar % arachidonic acid in liver lipid classes of guinea pig fed diets containing 10% black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7–8 animals ± SEM) with different letters are significantly different.

proportionately high compared with the diet content and may indicate a conservation of this nutritionally essential fatty acid.

The LA content of CL was higher than dietary content due to the special affinity of CL in mammalian tissue for 18-carbon fatty acids (37). Although the fatty acid profile of CL can be influenced by a number of factors such as aging (38), alcohol ingestion (39, 40), hyperthyroidism (41), fat-free diets (42, 43), it appears that, for the guinea pig, the low content of LA in the lard diet was not a treatment severe enough to depress significantly the incorporation of this fatty acid in liver CL. The LA content of cardiolipin may be protected because of its role in the integrity of the mitochondrial inner membrane (44–46) and in the electron transport system (47).

The stability of the levels of AA in liver lipids of the guinea pig observed in this experiment after variable LA intakes has also been noted in human plasma phospholipids (18, 48) and in guinea pig liver triglycerides and total phospholipids (19). The importance of maintaining the levels of this acid relates perhaps to its importance in membrane structure and as a precursor of the eicosanoids of the 2 series.

The ratio of DHLA/AA in the phospholipids of most species, including humans, is normally 1/3 to 1/50 (49) and this may be one reason why the synthesis of the dienoic eicosanoids dominates over that of the monoenoic. In this experiment, the DHLA/AA ratio in the guinea pig fed BCO was notably greater in every liver lipid class, as is also true in liver TG and phospholipids of guinea pigs fed evening primrose oil (19, 57).

This supports the idea (19) that, in the guinea pig, Δ5 desaturase becomes a limiting enzyme in the further transformation of GLA when it is supplied in the diet. If the synthesis of eicosanoids is partly regulated by substrate availability as has been suggested (22, 26), then the potential for increased synthesis or increased relative synthesis of the monoene eicosanoids compared with the diene eicosanoids is greater in the BCO fed group. the observation that feeding BCO, which contained SA, increased EPA content in liver

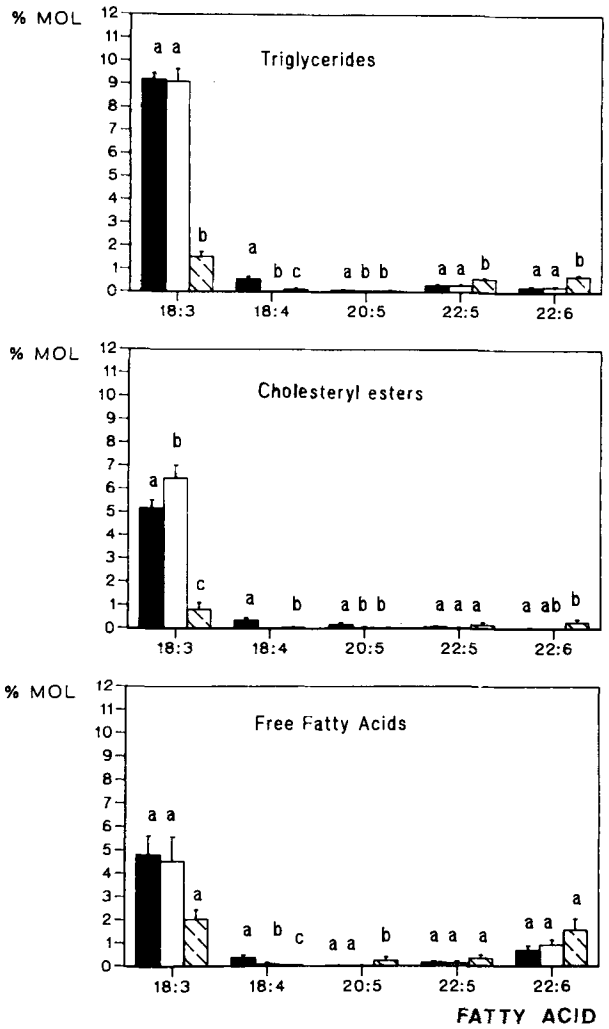

FIG. 5. n-3 fatty acids (mol %) in triglycerides (TG), cholesteryl esters (CE) or free fatty acids (FFA) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7–8 animals ± SEM) with different letters are significantly different.

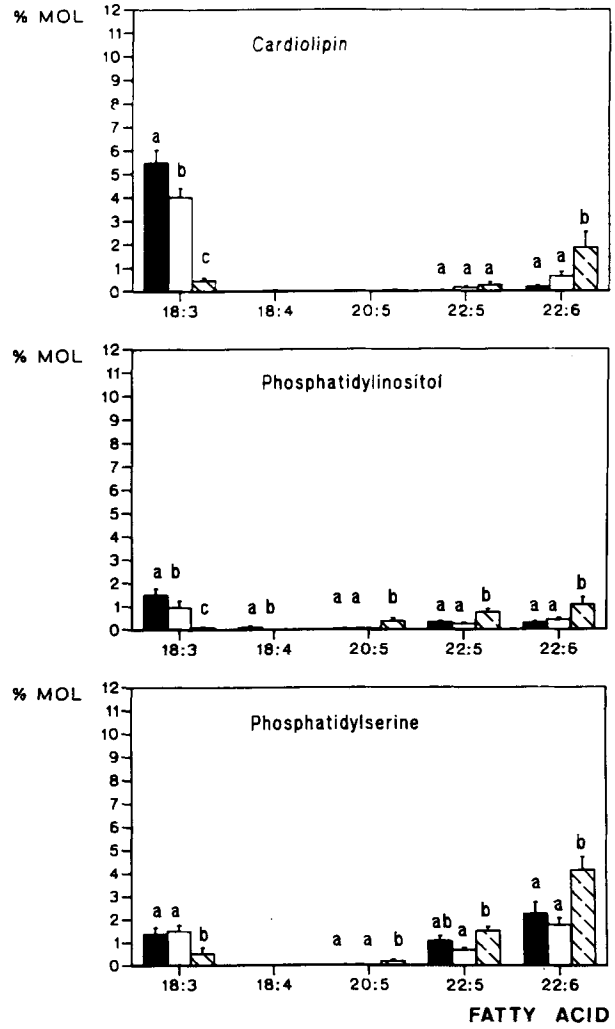

FIG. 6. n-3 fatty acids (mol %) in cardiolipin (CL), phosphatidylinositol (PI) or phosphatidylserine (PS) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7–8 animals ± SEM) with different letters are significantly different.

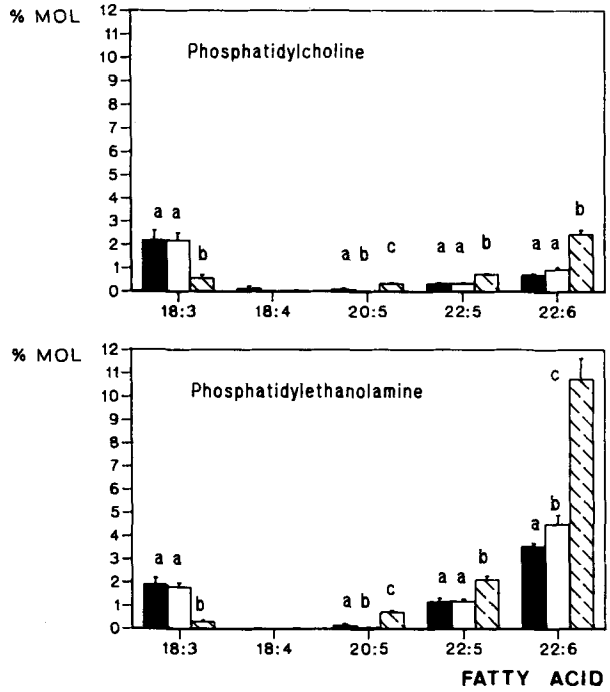

FIG. 7. n-3 fatty acids (mol %) in phosphatidylcholine (PC) or phosphatidylethanolamine (PE) in livers of guinea pigs fed black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). Values (means for 7-8 animals ± SEM) with different letters are significantly different.

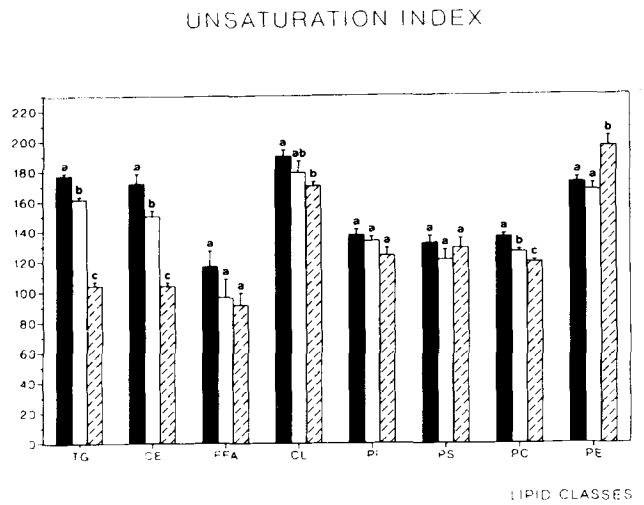

FIG. 8. The unsaturation index of the liver lipid classes from guinea pigs fed diets containing 10% black currant seed oil (dark bars), walnut oil (open bars) or lard (hatched bars). The unsaturation index is the sum of (a × b); a is the relative molar % of each unsaturated fatty acid, b is the number of double bonds for that particular fatty acid. Values (means for 7-8 animals ± SEM) with different letters are significantly different.

indicates that BCO feeding could possibly affect the synthesis of the triene eicosanoids in the guinea pig.

Ingested n-3 fatty acids were to a large extent transformed to higher chain analogues. This was noticeable in the BCO- and WO-fed groups of guinea pigs but was particularly evident in the lard-fed animals. A similar observation has been made in phospholipids of rat liver (50, 51) and muscle (52) and in human serum phospholipids (18).

Fatty acids of the different families compete for the same desaturases with an order of priority n-3 > n-6 > n-9 (53-55). When n-6 fatty acids are provided at low levels in the diet, lack of competition may permit desaturation of the n-3 fatty acids to occur at rapid rates. Gibson et al. (50) have remarked on the compensatory incorporation of DHA where dietary n-6 fatty acids are low.

This highly unsaturated fatty acid contributes to the maintenance of the unsaturation index, one determinant of membrane fluidity (56). When rats were fed diets containing fats of widely varying levels of unsaturation (50), liver mitochondrial and microsomal membrane phospholipid fatty acids maintained the same unsaturation index. In the present study, it was observed that, in the guinea pig, there was a tendency to maintain a constant unsaturation index, but differences were still found in many of the lipid classes, particularly the neutral lipids, generally in the direction to be expected based on dietary unsaturation.

In summary, the results of the present experiment demonstrated that feeding the guinea pig black currant seed oil resulted in important levels of incorporation of DHLA in liver lipid classes and higher ratios of DHLA/AA in all the lipid classes studied. The potential for increased or proportionately increased synthesis of the eicosanoids of the 1 series and possibly the 3 series compared with the 2 series is greater with black currant seed oil as a dietary lipid source than with the other two fats.

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REFERENCES

1. Willis, A.L., Comai, K., Kuhn, D.C., and Paulsrud, J. (1974) *Prostaglandins 8*, 509-519.
2. Silver, M.J., Smith, J.B., Ingerman, C., and Kocsis, J.J. (1975) *Prostaglandins 4*, 863-875.
3. Vargaftig, B.B., and Zirinis, P. (1973) *Nature (London) New Biol. 244*, 114-116.
4. Kernoff, P.B.A., Willis, A.L., Stone, K.J., Davies, J.A., and MacNicol, G.P. (1977) *Br. Med. J. 2*, 1441-1444.
5. Sim, A.K., and MacCraw, A.P. (1977) *Thromb. Res. 10*, 385-397.
6. Brenner, R.R. (1982) *Progr. Lipid Res. 20*, 41-48.
7. Ayala, S., Gaspar, G., Brenner, R.R., Peluffo, R.O., and Kunau, W. (1973) *J. Lipid Res. 14*, 296-305.
8. Darcet, P., Driss, F., Mendy, F., and Delhaye, N. (1980) *Ann. Nutr. Alim. 34*, 277-290.
9. Blond, J.P., Lemarchal, P., and Spielmann, D. (1981) *C.R. Acad. Sc. Paris 292* (16) serie III, 911-914.
10. Paccalin, J., Mendy, F., Costa, J.M., Lacomere, R.P., Delhaye, N., and Spielmann, D. (1981) *J. Physiol. Paris 77*, 42A.
11. Poisson, J.P., Lemarchal, P., and Blond, J.P. (1978) *Diabete Metab. 4*, 39-45.
12. Narce, M., Poisson, J.P., Divry-Aellen, M.T., and Chanussot, B. (1986) in *Les Malnutritions Dans Les Pays du Tiers-Monde* (Lemonnier, D., and Ingenbleek, Y., eds.) colloque INSERM, Vol. 136, pp. 229-236, INSERM, Paris.

13. Traitler, H., and Winter, H. (1986) *Prog. Lipid Res. 25*, 255-261.
14. Hoy, C.E., Holmer, G., Kaur, N., Byrjalsen, I., and Kirstein, D. (1983) *Lipids 18*, 760-771.
15. Cunnane, S.C., Manku, M.S., and Horrobin, D.F. (1985) *Br. J. Nutr. 53*, 441-448.
16. Cunnane, S.C., Manku, M.S., and Horrobin, D.F. (1985) *Br. J. Nutr. 53*, 449-458.
17. Stone, K.J., Willis, A.L., Hart, M., Kirtland, S.J., Kernoff, P.B.A., and McNicol, G.P. (1979) *Lipids 14*, 174-180.
18. Lasserre, M., Mendy, F., Spielmann, D., and Jacotot, B. (1985) *Lipids 20*, 227-233.
19. Huang, Y.S., Horrobin, D.F., Manku, M.S., and Mitchell, J. (1985) *Proc. Soc. Exp. Biol. Med. 178*, 46-49.
20. Lands, W.E.M., and Samuelsson, B. (1968) *Biochim. Biophys. Acta 164*, 426-429.
21. Vonkeman, H., and Van Dorp, D.A. (1968) *Biochim. Biophys. Acta 164*, 430-432.
22. Lands, W.E.M., Le Tellier, P.R., Rome, L.H., and Vanderhoek, J.Y. (1973) *Adv. Biosc. 9*, 15-28.
23. Samuelsson, B. (1982) in *Prostaglandins and Cancer: First International Conference* (Powles, T.J., Bockman, R.S., Honn, K.V., and Ramsell, P., eds.) pp. 1—20, Alan R. Liss, New York, NY.
24. Codde, J.P., Beilin, L.J., Croft, K.D., and Vandongen, R. (1985) *Prostaglandins 29*, 895-910.
25. Spector, A.A., and Yorea, M.A. (1985) *J. Lipid Res. 26*, 1015-1035.
26. Lands, W.E.M. (1979) *Annu. Rev. Physiol. 41*, 633-652.
27. Christiansen, K. (1979) *Anal. Biochem. 66*, 93-99.
28. Juaneda, P., and Rocquelin, G. (1985) *Lipids 20*, 40-41.
29. Kolarovic, L., and Traitler, H. (1985) *J. High Resolut. Chromatogr. Commun. 8*, 341-346.
30. Ledeen, R.W., Yu, R.K., and Eng L.F. (1973) *J. Neurochem 21*, 829-839.
31. Fishmann, P.H., Quarles, R.H., and Max, S.R. (1979) in *Densitometry in Thin Layer Chromatography* (Touchstone, J.C., and Sherma, J., eds.) pp. 315-327, Wiley, New York, NY.
32. Shehata, A.Y., deMan, J.M., and Alexander, J.C. (1970) *Can. Inst. Food Technol. J. 3*, 85-89.
33. Epps. D.E., and Kaluzny, M.A. (1985) *J. Chromatogr. 343*, 143-148.
34. Christie, W.W. (1976) in *Lipid Analysis. Isolation, Separation, Identification, and Structural Analysis of Lipids*, Pergamon Press, Oxford, U.K.
35. Traitler, H., and Kolarovic, L. (1983) *J. Chromatogr. 279*, 69-73.
36. Kolarovic, L., Traitler, H., and Ducret, P. (1984) *J. Chromatogr. 314*, 233-241.
37. Ioannou, P.V., and Golding, B.T. (1979) *Prog. Lipid Res. 17*, 279-318.
38. Keranen, A., Kankare, P., and Hallman, M. (1982) *Lipids 17*, 155-159.
39. Waring, A.J., Rottenberg, H., Ohnishi, T., and Rubin, E. (1981) *Proc. Natl. Acad. Sci. USA 78*, 2582-2586.
40. Cunningham, C.C., Filus, S., Bottenus, R.E., and Spach, P.I. (1982) *Biochim. Biophys. Acta 712*, 225-233.
41. Ruggiero, F.M., Landriscina, C., Gnoni, G.V., and Quagliariello, E. (1984) *Lipids 19*, 171-178.
42. Tischer, K., and Glenn, J.L. (1965) *Biochim. Biophys. Acta 98*, 502-511.
43. Stancliff, R.C., Williams, M.A. Ustumi, K., and Packer, L. (1969) *Arch. Biochem. Biophys. 131*, 629-642.
44. Rand, R.P., and Sengupta, S. (1971) *Biochim. Biophys. Acta 255*, 484-492.
45. De Kruijff, B., and Cullis, P.R. (1980) *Biochim. Biophys. Acta 602*, 477-490.
46. De Kruijff, B., Verkley, A.J., Van Echteld, C.J.A., Gerritsen, W.J., Mombers, C., Noordam, P.C., and De Gier, J. (1979) *Biochim. Biophys. Acta 555*, 200-209.
47. Fry, M., and Green, D.E. (1981) *J. Biol. Chem. 256*, 1874-1880.
48. Ferretti, A., Judd, J.T., Marshall, M.W., Flanagan, V.P., Roman, J.M., and Matusik, E.J. (1985) *Lipids 20*, 268-272.
49. Willis, A.L. (1984) in *Present Knowledge in Nutrition* (Olson, R.E., ed.) pp. 90-115, The Nutrition Foundation, Washington, D.C.
50. Gibson, R.A., McMurchie, E.J., Charnock, J.S., and Kneebone, G.M. (1984) *Lipids 19*, 942-951.
51. Iritani, N., Fujikawa, S. (1982) *J. Nutr. Sci. Vitaminol. 28*, 621-629.
52. Paturneau-Jouas, M., Durand, G., Nouvelot, A., Masson, M., and Bourre, J.M. (1982) *Reprod. Nutr. Develop. 22*, 193-200.
53. Brenner, R.R., and Peluffo, R.O. (1966) *J. Biol Chem. 241*, 5213-5219.
54. Holman, R.T. (1964) *Fed. Proc. 23*, 1062-1067.
55. Siguel, E.N., and Maclure, M. (1987) *Metabolism 36*, 664-669.
56. Awad, A.B. (1986) in *Advances in Human Nutrition* (Kabara, J.J., and Scherr, G.H., eds.) Vol. 3, pp. 1-14, Chem-Orbital, Park Forest, IL.
57. Huang, Y.S., and Horrobin, D.F. (1987) *Ann. Nutr. Metab. 31*, 18-28.

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