THE INFLUENCE OF DIETARY CANOLA OIL ON THE LEVEL OF VARIOUS POLYUNSATURATED FATTY ACIDS IN MEMBRANE PHOSPHOLIPIDS OF RAT BRAIN, SCIATIC NERVE, RETINA AND KIDNEY

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Summary

Alpha-linolenic acid /18:3 n-3; ALA/ and linoleic /18:2 n-6; LA/ are two major essential fatty acids found in Canola oil. Successive chain elongation and desaturation reactions of these two polyunsaturated fatty acids results in the formation of other long chain fatty acids. Of these, Dihomo- -linolenic acid /20:3 n-6; DHLA/, arachidonic acid /20:4 n-6; AA/, and eicosapentaenoic acid /20:5 n-3; EPA/ serve as presursors of eicosanoids. Decosahexaenoic acid /22:6 n-3; DHA/, a vital fatty acid in neural tissues is a potent competitive inhibitor of the conversion of AA to prostaglandins. In view of the importance of these fatty acids on eicosanoid biosynthesis, it was of interest to determine if Canola oil could affect the level of the n-3/n-6 fatty acids in membrane phospholipids of brain, sciatic nerve, retina, and kidney tissues of postweaning male rats. A significant decrease of AA in kidney phospholipids, and LA in all the neural tissues examined from the Canola-fed rats was observed. In Canola-fed groups, levels of EPA were increased in phospholipids of sciatic nerve, retina and kidney. However, no significant levels of EPA could be detected in the brain lipids. Significant increases in DHA were found in the brain and kidney phospholipids. ALA was not generally detected in any of the tissues examined. The results indicate that dietary Canola oil can modulate the level of precursors of eicosanoids in membrane phospholipids within a relatively short period of time in growing male rats. Increased concentrations of DHA seen in tissues of Canola oil fed rats may have important implications on the biosynthesis of prostaglandins, thromboxanes, and prostacyclins in addition to its membrane functions.

Introduction

Linoleic acid (18:2 n-6; LA) and alpha-linolenic acid (18:3 n-3; ALA) are considered as essential fatty acids in the diets of many mammalian species including the human (Burr and Burr, 1930; Lamptey and Walker, 1976; Yamanaka et al., 1981; Tinoco, 1982; Holman et al., 1982; Bjerve et al., 1987). The other long chain fatty acids of the n-6 and n-3 series are formed from LA (n-6) and ALA (n-3) respectively by successive chain elongation and desaturation reactions. Dihomo-γ-linolenic acid (18:3 n-6; DHLA), and arachidonic acid (20:4 n-6; AA), derived from LA are the precursors of prostaglandins, thromboxanes and prostacyclins of the 1- and 2- series and leukotrienes of the 3- and 4- series respectively. On the other hand, eicosapentaenoic acid (20:5 n-3; EPA) formed from ALA is converted to prostaglandins of the 3- series and leukotrienes of the 5-series. The eicosanoids derived from AA have been implicated in many pathological states. The effects of the n-3 dietary fatty acids on plasma and tissue phospholipid fatty acids are of interest for two reasons: a) administration of n-3 fatty acids depresses substantially the content of AA in tissues thereby limiting the amount of the available AA for the synthesis of eicosanoids, b) ALA, EPA and DHA are competitive inhibitors of cyclooxygenase, a key enzyme in the biosynthesis of prostaglandins.

Although many vegetable oils serve as important sources of LA, only the Canola oil can provide significant amounts of both LA and ALA. Such an oil containing high amounts of ALA, may be nutritionally adequate in view of the importance of ALA in the body including the neural, immune and blood clotting systems. In addition, DHA derived from ALA is considered to be essential for membrane functioning in neural tissues (Tinoco, 1982). The purpose of this study was to examine the effects of Canola oil, a rich source of ALA, in comparison with sunflower oil, a rich source of LA, on

phospholipid fatty acids as these serve as precursors of eicosanoids in selected tissues such as the brain, sciatic nerve, retina and kidney.

Experimental Procedures

Male weanling Sprague-Dawley rats, with an average weight of 63 g (High Oak Ranch, Goodwood, ONT) were randomly divided into two groups and fed *ad libitum* semi-purified, isocaloric diets containing either sunflower oil (18:2 n-6, 53.5%; 18:3 n-3, 0.2%) or Canola oil (18:2 n-6, 14.9%; 18:3 n-3, 7.1%). The compositions of the two diets are given in Table 1. The animals were housed individually in suspended steel cages at 25°C and at a relative humidity of 45%. Feed intakes and body weights were recorded for 5 weeks at which time the animals were anesthetized with methoxyfluorane (Metofane) and exsanguinated.

Chromatographic Analysis of lipids and fatty acids

The brain, sciatic nerve, retina, and kidney tissues were quickly removed, weighed, and immediately homogenized in 20 volumes of chloroform: methanol (2:1, v/v) per g wet weight tissue (Folch et al., 1957). The resulting tissue lipid extracts were fractionated into phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) by thin-layer chromatography on pre-coated silica gel H plates (E. Merck, Darmastadt, FRG) using a chloroform: methanol: acetic acid: water solvent system (50: 37.5: 3.5:2, v/v/v/v) (Mahadevappa and Holub, 1986). The bands corresponding to the above phospholipids were scraped from the plates following detection by 2,7-dichlorofluorescein, and transmethylated with 6% sulphuric acid in methanol (v/v) in the presence of monopentadecanoin, an internal standard fatty acid (Mahadevappa and Holub, 1982). The unknown fatty acid methyl esters were analyzed isothermally in a Hewlett-Packard model 5890A gas chromatograph, equipped with a DB-225 megabore column (Chromatographic

Specialties, Brockville, ONT). Identification of the unknown fatty acids was accomplished by a comparison of their retention times with those of known fatty acid methyl ester standards. The results from these chromatographic runs, expressed as % weight of the total fatty acids.

Results

No significant differences in the cumulative weight gains and daily average food intakes of the sunflower and Canola groups were observed. The estimated intakes of ALA were 3.4 and 140.6 mg/day, and the LA intakes 1.0 and 0.3 g/day for the sunflower and Canola groups, respectively during the final week of the experiment.

Administration of ALA-rich Canola oil to the rats for five weeks resulted in several changes in the n-6 and n-3 fatty acids across the individual phospholipid fractions (PC, PE, PS, and PI) from the brain, sciatic nerve, retina, and kidney. Little or ALA (18:3 n-3) was detectable in any of the phospholipid fractions of the above tissues from rats receiving either a diet containing sunflower or Canola oil (data not shown). However, levels of DHA, a product of ALA metabolism were significantly elevated in PE and PS of the brain tissue and in PC, PE, PS, and PI of the kidney from rats receiving Canola oil (Figs. 1 and 2). EPA, another product of ALA and a precursor of the 3-series prostaglandins and the 5-series leukotrienes, also appeared in some phospholipid fractions of the sciatic nerve, retina (data not shown), and kidney of the Canola group (Fig. 2). However, no marked changes were observed in the amounts of DHA in the various phospholipid fractions of the retinal tissue (Fig. 3). Furthermore, the reduction of AA by dietary Canola oil was less pronounced in brain and retinal phospholipids (Figs. 1 and 3), and more pronounced in PC and PS fractions of the kidney (Fig. 4). On the other hand, the levels of LA and 22:5 n-6 were markedly lower in phospholipids of the brain,

sciatic nerve, and retinal tissues from rats given a Canola oil containing diet relative to those of a sunflower oil diet (data not shown).

Discussion

Our results demonstrate that dietary Canola oil containing high levels of ALA can affect phospholipid fatty acid compositions of neural tissues and kidney. Although little or no ALA could be detected in these tissues of rats given a Canola oil-containing diet, significant increases in DHA as seen in this study are consistent with previous findings in the rat when ALA was consumed (Hoy et al., 1983; Tarozzi et al., 1984; Carlson et al., 1986; Anding and Hwang, 1986). These results suggest that dietary ALA is rapidly desaturated and elongated by neural tissues and the kidney and/or by other tissues (e.g., liver) prior to assimilation of the ALA derivatives. In the present study, the AA content in phospholipids of neural tissues including the brain, sciatic nerve, and retina was least affected suggesting that the level of Canola ALA used may have very limited effects of AA formation (Anding and Hwang, 1986). However, the extent of impaired formation of AA may have been much greater in the kidney, where a marked reduction of AA was observed. This differential effect of ALA may be attributed to its selective effects depending upon the type of tissue studied. Marked elevations of EPA in phospholipids from the sciatic nerve, retina, and kidney, and DHA in major phospholipids from the brain observed in rats given a diet containing Canola oil relative to sunflower oil, could contribute to the inhibition of cyclooxygenase thereby limiting the production of AA- derived eicosanoids (Pace-Asciak and Wolfe, 1968; Needleman et al., 1979; Hwang and Carrol, 1980; Marshall et al., 1983; Corey et al., 1983; Adam et al., 1986; Zollner, 1986). The reduction in the other n-6 fatty acids (18:2 and 22:4) in phospholipids of neural tissues and the kidney by dietary Canola

oil (data not shown) support the findings of the Foot et al., (1982) in the synaptosomal membrane and provide further evidence that dietary fatty acid intakes can affect phospholipid fatty acid composition of neural tissues and kidney within a relatively short time in the post-weaning rat.

In conclusion, our results imply that dietary Canola oil, which contains significant amounts of ALA can modulate the levels of precursors of eicosanoids such as AA and EPA in neural tissues and the kidney and, also, can increase the content of DHA, a potent inhibitor of cyclooxygenase in tissue phospholipids. As ALA does not accumulate in these tissues, it can be concluded that the potential effects that Canola oil containing ALA may have on the biosynthesis of eicosanoids are possibly mediated through a combination of the reduction of the precursor AA in tissue phospholipids and the competitive inhibition of cyclooxygenase by increased amounts of EPA and/or DHA, both of which are derived from ALA. Dietary Canola oil can also provide for the enrichment of membrane phospholipids from brain tissue in the functionally-important DHA.

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Table 1

Composition of the Experimental Diets.

Ingredient (wt %)	Sunflower	Canola
Vitamin-free casein	20.0	20.0
Choline chloride	0.2	0.2
Corn starch	10.0	10.0
Sucrose	50.0	50.0
Vitamin mixture	1.0	1.0
Salt mixture	4.5	4.5
Alpha Floc	4.[illegible]	4.4
Fat	10.0	10.0
Antioxidant (Santoquin)	0.005	0.005

a) Canola oil obtained from Canadian Vegetable Oil Processing, Hamilton, ONT

b) Vitamin mixture contained (μg/g diet): 6μg B_1; 6μg B_2; 7μg B_6; 30 μg nicotinic acid; 32μg Ca pantothenate; 2μg folic acid; 0.2 μg d-biotin; 0.1 μg B; 1000 μg i-inositol; 8 μg A; 2.5 μg D; 0.02 μg E; 0.5 μg K.

c) Salt mixture contained (mg/100 g diet): 720 mg $CaCO_3$; 5.3 mg $CuSO_4 \cdot 5H_2O$; 1130 mg CaH_2PO_4; 63.5 mg Fe citrate; 230 mg $MgSO_4$; 730 mg KCl; 0.3 mg KI; 5.3 mg $ZnCO_3$; 600 mg NaH_2PO_4; 1.0 mg Cr-acetate; 8.4 mg $ZnSO_4$; 29.3 mg $MgCl_2$; 2.7 mg MnCl; 0.02/μg Na selenite.

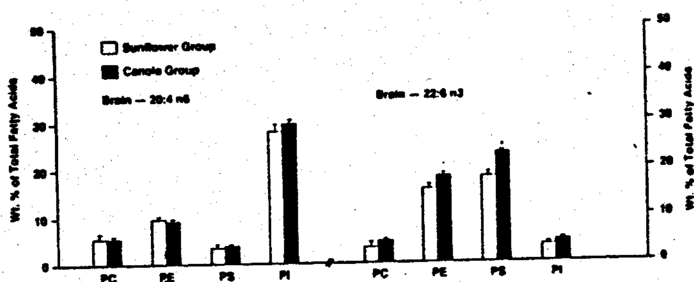

Fig. 1. The open and closed bars represent % wt of AA or DHA in individual phospholipids of the brain from rats fed diets containing sunflower oil and Canola oil, respectively. Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine, PI, phosphatidylinositol.

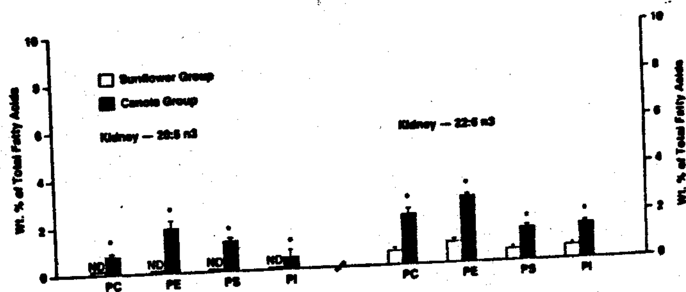

Fig. 2. The open and closed bars represent % wt of EPA or DHA in individual phospholipids of the kidney from rats fed diets containing sunflower oil and Canola oil, respectively. Abbreviations: Same as in Fig. 1.

Fig. 3. The open and closed bars represent % wt of AA or DHA in individual phospholipids of the retina from rats fed diets containing sunflower oil and Canola oil respectively. Abbreviations: Same as in Fig. 1.

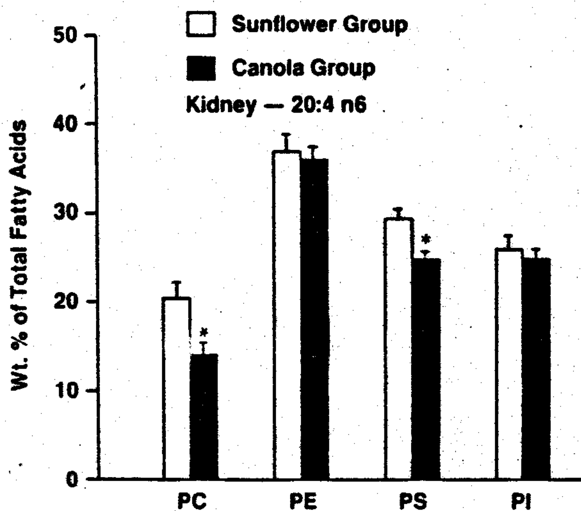

Fig. 4. The open and closed bars represent % wt of AA in individual phospholipids of the kidney from rats fed diets containing sunflower oil and Canola oil respectively. Abbreviations: Same as in Fig. 1.

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