

## Modulation of lipids of brain, heart and liver by dietary rapeseed oil as compared to olive oil and high-oleic sunflower oil

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### Abstract

The level of the cardioprotective  $\omega$ 3 docosahexaenoic acid was steeply increased in total lipids, phosphatidylethanolamines and phosphatidylcholines of heart and liver, but not of brain, of rats after feeding rapeseed and olive oils as compared to high-oleic sunflower oil. The concentration of arachidonic acid ( $\omega$ 6), a precursor of "inflammatory" eicosanoids, was lowered in cardiac lipids after feeding rapeseed oil. The changes in the levels of the  $\omega$ 3 and  $\omega$ 6 fatty acids are attributed to a relatively high proportion of  $\alpha$ -linolenic acid ( $\omega$ 3) in rapeseed oil and phenolic constituents of olive oil. Dietary rapeseed and olive oils seem to be beneficial to health for the prevention of arteriosclerosis.

### Introduction

The incidence of coronary heart disease in the Mediterranean countries is relatively low which is attributed, in part, to the high amount of the monounsaturated oleic acid (*cis*-9-octadecenoic acid) present in olive oil in Mediterranean-type diet (Nestle, 1995). It has been shown that oleic acid can be as effective as polyunsaturated fatty acids in lowering low density lipoprotein cholesterol of blood plasma (Gardner and Kraemer, 1995; Mattson and Grundy, 1985; Riccardi and Rivellese, 1993). The mechanism by which oleic acid lowers the incidence of coronary heart disease is not known, so far. The influence of minor components of plant oils, such as phenolics and flavonoids, on fatty acid metabolism has been taken into consideration to explain the physiological effects of olive oil (Petroni et al., 1994).

The present study was undertaken to establish whether the beneficial effects of dietary olive oil are solely due to the high levels of oleic acid. In order to achieve this, oils with high and intermediate levels of oleic acid were compared with olive oil. We report here the effects of dietary 'double-zero' medium-oleic rapeseed oil (RAP), olive oil (OLI) and high-oleic sunflower oil (HOS) on fatty acid alterations in total lipids, phosphatidylethanolamines and phosphatidylcholines of heart, liver and brain of rats. In particular, the n-3 and n-6 long-chain polyunsaturated fatty acids (LC-PUFA) in the two phospholipid classes were examined to determine whether the levels of these fatty acids were modulated by the concentrations of the corresponding precursor fatty acids of diet or whether dietary oleic acid itself had some modulating effects on the fatty acid composition of the cellular phospholipids.

### Materials and methods

Iso-caloric pelleted diets (metabolizable energy 13.1 kJ/g) containing recommended levels of proteins, carbohydrates, vitamins and nutrient minerals as used in the Altromin standard diet for rats and one of the above experimental oils (120 g/kg diet) plus corn oil (20 g/kg diet) were prepared by Altromin International, Lage, Germany (Weber et al., 1995). Composition of the major constituent

fatty acids of the dietary triacylglycerols and the respective oils of the three groups was as follows: Rapeseed oil, RAP (7 and 6% 16:0, 54 and 60% 18:1 n-9, 28 and 22% 18:2 n-6, 7 and 8% 18:3 n-3), olive oil, OLI (14 and 12% 16:0, 66 and 74% 18:1 n-9, 17 and 11% 18:2 n-6, 0.5 and 0.6% 18:3 n-3); high-oleic sunflower oil, HOS (5 and 6% 16:0, 75 and 82% 18:1 n-9, 15 and 9% 18:2 n-6, <0.2 and <0.2% 18:3 n-3).

Weaned male Wistar rats (Lippische Versuchstierzucht, Extertal, Germany) weighing 85 - 90 g were caged individually and divided into groups of 10 animals. The rats were fed the diets containing rapeseed oil, olive oil or high-oleic sunflower oil for 10 wk. The rats were killed by subjecting them to ether narcosis followed by sectioning of the aorta. Heart, liver and brain were rapidly removed and kept frozen until the lipids were extracted. All procedures for the animal experiments were approved by the official commission for animal experimentation [Der Regierungspräsident Münster, permission no. 26.0834 (48/90)].

Procedures used for lipid extraction, fractionation of lipid classes by thin-layer chromatography, determination of fatty acid composition of total lipids and lipid classes by gas chromatography of methyl esters and statistical analysis are reported elsewhere (Weber and Mukherjee, 1998).

## Results and discussion

The composition of the major constituent fatty acids in total lipids of hearts and livers of individual rats after the feeding of rapeseed oil (RAP), olive oil (OLI) or high-oleic sunflower oil (HOS) diets over a period of ten weeks is given in Table 1 (Weber and Mukherjee, 1998). These results show that the proportions of docosahexaenoic acid (DHA) in total lipids of heart were significantly higher ( $P<0.01$ ) in OLI and RAP than in HOS. Moreover, DHA concentrations of total liver lipids were significantly higher for the groups RAP ( $P<0.01$ ) and OLI ( $P<0.05$ ) than HOS (Table 1).

Concentration of arachidonic acid (AA) in total lipids of heart of the RAP group was significantly lower than in OLI and HOS groups, whereas in the total lipids of liver the proportions of AA were essentially identical for the three groups (Table 1). The proportions of linoleoyl moieties were significantly higher in total lipids of heart and liver of the RAP group as compared to the other two groups (Table 1).

In the total lipids of brain no significant difference was observed between the three groups with respect to the levels of AA and DHA. However, the proportion of n-6-LC-PUFA (AA + 22:4 n-6 + 22:5 n-6) in total brain lipids was significantly lower for the RAP group than OLI and HOS groups.

The fatty acid compositions in the pooled samples of phosphatidylethanolamines (PE) of heart and liver of rats fed RAP, OLI and HOS diets are given in Table 2 (Weber and Mukherjee, 1998). The proportions of DHA in the PE fractions of heart of both RAP and OLI groups were increased by about 4.5- and 3-fold, respectively, as compared to the HOS group, whereas the proportions of AA in the PE of hearts were similar (20 - 25%) in all the three groups (Table 2).

The proportions of AA were similar (29 - 33%) in the pooled samples of PE of the livers of all three experimental groups, whereas a steep increase (by about fourfold) in the proportions of DHA was found in this lipid class isolated from the livers of both RAP and OLI groups as compared to PE of the HOS group (Table 2).

**Table 1:** Composition (%) of the major constituent fatty acids in total lipids of hearts and livers of individual rats after feeding rapeseed oil (RAP), olive oil (OLI) and high-oleic sunflower oil (HOS) for ten weeks\*

Fatty acids	Fatty acid composition (%) of total lipids of							
	Heart				Liver			
	RAP	OLI	HOS	± SEM	RAP	OLI	HOS	± SEM
16:0	9.6 <sup>a</sup>	9.1 <sup>a</sup>	9.5 <sup>a</sup>	1.4	14.8 <sup>b</sup>	17.9 <sup>a</sup>	17.5 <sup>a</sup>	1.5
18:0	19.1 <sup>b</sup>	21.7 <sup>a,b</sup>	25.2 <sup>a</sup>	3.9	11.1 <sup>b</sup>	9.8 <sup>b</sup>	15.5 <sup>a</sup>	2.9
18:1 n-9	17.5 <sup>a</sup>	12.1 <sup>a</sup>	16.3 <sup>a</sup>	5.5	27.7 <sup>b</sup>	38.9 <sup>a</sup>	31.9 <sup>b</sup>	6.3
18:1 n-7	4.7 <sup>a</sup>	4.2 <sup>a</sup>	3.0 <sup>b</sup>	0.6	1.5 <sup>a</sup>	0.7 <sup>b</sup>	1.7 <sup>a</sup>	0.3
18:2 n-6	16.8 <sup>a</sup>	12.6 <sup>b</sup>	12.7 <sup>b</sup>	1.7	18.6 <sup>a</sup>	11.0 <sup>b</sup>	8.8 <sup>c</sup>	2.0
18:3 n-3	0.9	Tr	Tr <sup>***</sup>	0.2	1.8	Tr	Tr	0.3
20:4 n-6	16.5 <sup>b</sup>	25.8 <sup>a</sup>	22.8 <sup>a</sup>	3.5	14.6 <sup>a</sup>	13.9 <sup>a</sup>	16.9 <sup>a</sup>	3.5
22:5 n-6	0.2 <sup>c</sup>	3.3 <sup>b</sup>	4.5 <sup>a</sup>	0.9	0.2 <sup>c</sup>	0.6 <sup>b</sup>	1.3 <sup>a</sup>	0.3
22:6 n-3	9.3 <sup>a</sup>	7.5 <sup>a</sup>	2.8 <sup>b</sup>	2.1	4.9 <sup>a</sup>	2.8 <sup>b</sup>	2.0 <sup>b</sup>	1.0

\*Values given are means ± pooled SEM (n=10). Values of fatty acids in the various feeding groups not carrying the same superscript are significantly different (P < 0.01).

The ratios of total n-3 LC-PUFA (predominantly DHA) to n-6 LC-PUFA (predominantly AA) in the PE of heart and liver of the HOS group were distinctly different from those of both OLI and RAP groups. Thus, in the PE of hearts the ratio of n-3 to n-6 was about 0.2 in the HOS group, 0.6 in the OLI group and 1.5 in the RAP group. The ratio of n-3 to n-6 was as low as 0.1 in the PE of livers of the HOS group, whereas in those of the RAP and OLI groups ratios of about 0.5 and 0.4, respectively, were observed.

In other organs and tissues, such as pooled samples of aorta, kidneys, lungs, spleen, stomach and jejunum as well as in blood higher proportions of DHA were found in the PE of rats fed the RAP and OLI diets than in those fed the HOS diet, however, the differences were much lower compared to those observed in heart and liver.

In the PE of brain, however, no significant difference was observed between the three groups with respect to the levels of AA, DHA as well as total n-6-LC-PUFA (AA + 22:4 n-6 + 22:5 n-6).

In the pooled samples of heart and liver lipids distinctly higher proportions of DHA were also found in the phosphatidylcholines (PC) of rats fed the RAP and OLI diets than in those fed the HOS diet (Table 3), however, the differences were much lower compared to those observed in PE of heart and liver of the animals of the corresponding experimental groups (Table 2) (Weber and Mukherjee, 1998).

The ratio of n-3 to n-6 LC-PUFA in the PC of heart and liver of the groups RAP and OLI was distinctly higher than that of the HOS group. The pattern of these ratios was similar to that observed for PE of the corresponding tissues, however, much less pronounced. The levels of AA in PC of

heart and liver were, however, distinctly higher for the OLI group as compared to RAP and HOS groups (Table 3) which did not reflect the levels of dietary 18:2 n-6 – a precursor of AA.

In the PC of brain no significant difference was observed between the three groups with respect to the levels of AA and total n-6-LC-PUFA (AA + 22:4 n-6 + 22:5 n-6). However, the proportion of DHA in PC of brain was significantly higher for the RAP group than the HOS group although there was no significant difference in the level of DHA in the PC of brain of the OLI and HOS groups.

**Table 2:** Fatty acid composition of phosphatidylethanolamines in hearts and livers of rats after feeding rapeseed oil (RAP), olive oil (OLI) and high-oleic sunflower oil (HOS) for ten weeks

Fatty acids	Fatty acid composition (%) of phosphatidylethanolamines of					
	Heart			Liver		
	RAP	OLI	HOS	RAP	OLI	HOS
16:0	4.7	4.8	7.0	10.9	12.4	18.9
18:0	25.3	23.4	33.3	26.5	25.4	32.2
18:1 n-9	5.8	6.1	8.6	4.4	4.8	5.4
18:1 n-7	2.1	1.8	1.1	2.0	1.7	0.8
18:2 n-6	2.8	1.7	2.0	4.4	2.3	2.6
20:4 n-6	19.9	24.5	24.9	30.2	32.5	29.1
22:4 n-6	1.3	3.1	2.7	0.1	1.1	1.4
22:5 n-6	0.2	9.7	11.7	Tr	2.5	4.4
22:5 n-3	6.1	1.8	0.5	2.5	1.2	0.3
22:6 n-3	31.7	23.0	7.4	17.1	14.8	3.9

In the RAP group the increase in DHA in the total lipids (Table 1) and PE (Table 2) of heart and liver can be easily explained by the presence of moderate proportions of  $\alpha$ -linolenoyl moieties in the RAP diet which are finally converted by a sequence of elongation/ desaturation/retroconversion reactions to DHA (Bourre et al., 1993; Sprecher et al., 1995; Winters et al., 1994). However, in the OLI group, in which the diet contained only trace amounts of  $\alpha$ -linolenic acid, some other constituents of the oil, such as phenolics and flavonoids (Petroni et al., 1994) seem to be responsible for the higher proportions of DHA found in the PE of heart and liver (Table 2).

It is of great interest that a large increase in DHA, which was found predominantly in PE after feeding of both the OLI and RAP diets (Table 2), was in heart, which is an organ affected particularly by atherosclerosis. These findings seem to contribute to the proposed protective effects of olive oil as well as plant oils containing  $\alpha$ -linolenic acid, such as rapeseed oil and soybean oil (Chan et al., 1991; Katan et al., 1995; Valsta et al., 1992), against atherosclerotic lesions, particularly in the heart.

**Table 3:** Fatty acid composition of phosphatidylcholines in hearts and livers of rats after feeding rapeseed oil (RAP), olive oil (OLI) and high-oleic sunflower oil (HOS) for ten weeks

Fatty acids	Fatty acid composition (%) of phosphatidylcholines of					
	Heart			Liver		
	RAP	OLI	HOS	RAP	OLI	HOS
16:0	12.4	10.3	12.4	18.6	16.1	19.2
18:0	27.2	28.7	32.6	23.2	24.9	28.2
18:1 n-9	6.3	5.8	7.7	5.4	6.4	7.2
18:1 n-7	4.5	3.2	2.9	2.7	2.2	1.3
18:2 n-6	7.2	3.7	3.6	9.7	4.6	4.5
20:4 n-6	31.3	40.4	32.4	31.1	36.1	31.5
22:4 n-6	0.5	1.3	1.2	Tr	0.4	0.4
22:5 n-6	Tr	1.5	2.1	Tr	1.3	1.7
22:5 n-3	3.4	1.0	0.4	0.9	0.6	Tr
22:6 n-3	6.1	3.4	1.1	6.1	5.8	1.8

It is envisaged that some of the health effects of olive oil upon cardiovascular diseases are due to improvement of heart function by DHA-induced changes of membrane structure (McLennan and Dallimore, 1995). DHA is known to be an important component of the structural lipids, e.g. PE, of cell membranes, predominantly of heart, liver, brain, and retina (Neuringer and Connor, 1986; Tahin et al., 1981).

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