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Effect of dietary oils containing graded amounts of 18:3 n-6 and 18:4 n-3 on cell plasma membranes

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Rats were fed diets containing a constant supply of lipids (10% by weight) differing from one another in the content of oleic acid (18:1 n-9), linoleic acid (18:2 n-6), and most peculiar 18-carbon polyunsaturated fatty acids, such as gamma-linolenic (18:3 n-6), alpha-linolenic (18:3 n-3), and stearidonic acid (18:4 n-3). Heart plasma membrane fatty acid composition from rats fed the different diets was affected. The diet containing the highest oleic acid content (olive oil diet) and the lowest PUFA content produced a significant decrease of linoleic acid content, while the oleic acid content was significantly lower when diets contained the lowest oleic acid percentage (black currant oil diet). Similarly, liver plasma membrane fatty acid composition was influenced by the different diets; in particular a significant higher content of the 20:4 n-6 was observed when rats were fed diets containing black currant oil (alone or into a 1:1 mixture with olive oil). Finally, the three diets tested influence only to a low level of significancy the fatty acid composition of the brain plasma membrane. Accordingly, the specific activity of 5'-nucleotidase, a typical cell plasma membrane enzyme, was not affected by diets neither in brain, nor in heart membrane preparations. On the contrary, the 5'-nucleotidase activity was highest in the membrane of liver cells from rats fed olive oil. A correlation between 5'-nucleotidase activity and membrane fatty acid composition cannot be stated in the conditions examined, however, possible biochemical mechanisms as the basis of 5'-nucleotidase behavior are discussed. (J. Nutr. Biochem. 6:21–26, 1995.)

Keywords: dietary lipid; gamma linolenic acid; stearidonic acid; plasma membrane; 5'-nucleotidase

Introduction

The influence of dietary fats on membrane lipid composition has been widely investigated in recent years. It has been demonstrated that fatty acid composition can be profoundly modified by the diet and that many membrane-associated functions can be altered by diet-induced changes in lipid composition of membranes.[1–4] Most studies have been performed using extremely unlikely diets in order to show a clear-cut effect of dietary intake on the chemical, physical and biochemical features of membranes.[5–8] The aim of this study is to verify whether the supplementation of lipids on the diets with olive oil and black currant oil taken in different proportions can influence the membrane lipid composition and whether the effect is tissue-specific.

The reason the two oils were chosen is because of their tremendously different gamma-linolenic (C18:3 n-6) and stearidonic (C18:4 n-3) acid contents. These two components show a peculiar position within the metabolism of fatty acids, since both are products of delta 6-desaturase, a key enzyme in the biosynthesis of polyunsaturated fatty acids and eicosanoids in animals.[9–11] Long chain polyunsaturated fatty acids and eicosanoids, particularly prostaglandins, leukotrienes, and tromboxanes are compounds playing essential roles in biochemical and physiological processes; therefore, a balanced pattern of eicosanoids precursors within cells and in extracellular fluids is of particular importance.[12–14] For instance, the delta-6-desaturase activity is highly sensitive to aging,[9,15] therefore it might be

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important to supply aged animals diets containing products of delta-6-desaturase in order to maintain the correct pattern of long chain PUFA and derivatives.

Although it is not the aim of the present study to investigate the effects of dietary lipids on aging, we believe that peculiar fatty acids such as 18:3 n-6 and 18:4 n-3, not still thoroughly experimental, are of particular interest for further study. This is intended to be preliminary with respect to investigations of such 18 carbon polyunsaturated fatty acids (18 C-PUFA) metabolism in aging. Dietary intake of these fatty acids during the rat's life in which delta-t-desaturase activity is beginning to decrease might result in significant changes of lipid metabolism. This study has been carried out in order to verify the influence of the dietary oils described above on cell plasma membrane fatty acid composition and on the activity of the membrane-bound 5'-nucleotidase. In this paper we report the results obtained supplying diets characterized by the content of graded amounts of delta-6-desaturase products, 18:3 n-6 and 18:4 n-3, to adult rats (15 months old).

Methods and materials

Analytical grade solvents (Carlo Erba, Milano, Italy) were used for the extraction of lipids. All biochemical reagents and enzyme substrates were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO USA).

Animals

Three groups (8 per group) of male Wistar rats weighing initially 500 g were purchased from Charles River, Italia. They were housed in individual cages at room temperature (15 to 25° C) in an approved animal facility. Animals had food and water ad libitum.

Diets

The composition of 100 g of fat-free diet was: casein 24 g, sucrose 18 g, starch 38 g, salt mixture 4 g, cellulose 4 g, choline chloride and vitamins 0.1 g, and water.

Each basic experimental diet was supplemented with 10% by weight of either olive (O), black currant (BC), or a 1:1 mixture of olive and black currant oil (OBC) as sources of increasing delta-6-desaturase products, respectively. Diets were made weekly and stored at −20° C. The fatty acid composition of each diet is shown in *Table 1*. Three groups of 8 rats each were fed these diets.

Membrane fractionation

After feeding the experimental diet for 3 months, the animals were weighed and sacrificed by decapitation.

The heart, liver, and brain were removed and thoroughly rinsed with 0.25 M sucrose, 1 mM EDTA, and 10 mM Tris-HCl, pH 7.4, to remove all traces of blood. The plasma membrane–enriched fraction was prepared essentially according to Nathan and Touster.[16] Briefly, tissues were homogenized in four volumes of buffer (0.25 M sucrose, 10 mM Tris/Cl, 1 mM EDTA, 0.4% fatty free bovine serum albumin, pH 7.2) in a tight fitting Potter-Elvejehm homogenizer. The crude extract was centrifuged (1,600*g* for 10 min). The supernatant was collected and centrifuged at 3,000*g* for 15 min. The pellet was washed with the same buffer and centrifuged at 12,000*g* for 5 min. The pellet was collected and suspended in 0.25 M sucrose, 10 mM Tris-Cl, pH 7.6, and either analyzed immediately or stored at −80° C. This preparation is referred to as a plasma membrane fraction. This preparation is 6-fold enriched in 5'-nucleotidase activity as compared with the crude homogenate and contains some contamination of mitochondria (The succinate:ubiquinone oxidoreductase activity was below 0.12 μmol/min/mg of protein in all the membrane-enriched preparations examined) and of microsomes. (The rotenone-insensitive NADH-cytochrome c reductase activity was below 0.15 μmol/min/mg of protein.)

Table 1 Fatty acid composition of the experimental diets

Fatty acid	O	OBC	BC
16:0	13.86	17.76	9.27
16:1	1.29	0.91	0.81
18:0	3.72	2.66	2.09
18:1	72.87	44.84	16.57
18:2 n-6	7.16	24.58	41.85
18:3 n-6	—	7.50	13.84
18:3 n-3	1.09	6.57	12.92
18:4 n-3	—	1.27	2.64

Values represent the average obtained from at least three separate extractions and analysis of the respective diets and are relative amounts, expressed as percentage (mol) of the total identified fatty acids.

The phospholipid and glyceride contents of plasma membrane isolated from animals fed different diets were comparable. Over 90% of the fatty acids of our extracts were coming from the phospholipid fraction. Protein concentration of the plasma membrane samples was determined by the biuret method, according to Gornall et al.[17]

Enzyme assays

The 5'-nucleotidase (EC 3.1.3.5) activity of plasma membrane was assayed under the initial velocity conditions either by a coupled assay according to Ipata[18] or by measuring spectrophotometrically the inorganic phosphate released from AMP during the reaction.[19] According to the latter method, 600 μl of the reaction mixture contained 10 mM $MgCl_2$, 100 mM glycine, pH 9.1, and 5 mM AMP (30° C); the reaction was started by addition of 1 mg of protein and stopped 10 min later adding 60 μl of 50% trichloroacetic acid.

The specific activity of our preparations ranged between 12 and 42 U/mg. One U/mg is referred to as 1 mmol/min/mg of protein. Succinate:ubiquinone oxidoreductase (Complex II; EC 1.3.5.1.), a marker enzyme for mitochondrial contamination, was measured by determining the reduction of coenzyme Q_1 by succinate (20). Rotenone-insensitive NADH-cytochrome c reductase, a marker enzyme for microsomal contamination, was evaluated by following the reduction of oxidized cytochrome c according to Siler Masters et al.[21]

Lipid analysis

Plasma membrane lipids were extracted by the method of Folch et al.[22] Fatty acids were methylated according to Stoffel et al.,[23] and the composition analysis was performed on a Varian model 3700 gas-chromatograph equipped with a glass column (2 m × 1.4 mm i.d.) filled with 15% DEGS on 80/100 mesh Gaschrom P at 200° C with N_2 as carrier gas at a flow rate of 30 ml/min.

The gas chromatographic peaks were identified on the basis of their retention time ratios relative to methyl stearate predetermined

on authentic samples. Gas-chromatographic traces and quantitative evaluations were obtained using a Spectra Physic computing integrator. The fatty acid composition of membranes represent that from total lipids. Of this the portion from phospholipids accounts for more than 90%.

Statistical analysis

Significant differences of mean values for fatty acid content and enzyme activity between the dietary treatment groups were determined by a one-way analysis of variance and a Student Newman Kuels test.[24]

Results and discussion

All animals appeared healthy after 3 months of feeding the experimental diets. Dietary lipid treatment had no significant effect on body, liver, brain, and heart weights.

Administration of lipids to the rats as in our experimental conditions had a somewhat low effect on the fatty acid profiles of plasma membranes from liver, brain, and heart (for comparison see Refs. 1, 23, 25, 26).

Effect of dietary olive and black currant oils on liver plasma membrane fatty acid profile

The three diets are characterized by a different content of oleic acid, linoleic acid, and delta-6-DPs (*Table 1*); the fatty acid content of liver plasma membrane is affected by these dietary intakes. *Table 2* shows the pattern of total membrane fatty acids for livers of rats fed the three diets examined. It is evident that 18:1 changes consistently from 20% in the O diet to about 15 in the OBC diet to 10.5 in the BC condition. The level of total n-6 acids is correlated with the amount of dietary linoleate as previously reported,[1] but membrane linoleic acid itself does not change significantly among treatments, although its level is consistently different in our diets. This suggests an active metabolism of 18:2 n-6, which is confirmed by the increasing level of 20:3 n-6 and 20:4 n-6. However, the difference in the amount of arachidonate between the OBC and the BC condition was not statistically significant. Finally, the different content of 18:4 n-3 in the three diets was reflected in significant differences of 22:5 n-3, though the level of this acyl group remains below 1% in any case.

Effect of dietary olive and black currant oils on the plasma membrane fatty acid profile of heart

Table 3, besides a general behavior of 18:1 and 18:2 n-6 reflecting their content in the diet, shows a statistically significant increase of 20:3 n-6, 20:5 n-3, and 22:5 n-3 and a slight decrease of 22:6 n-3 when BC is present in the diet. If one compares the profile of the heart with that of the liver, one can observe that in the liver, the BC oil diet leads to an increase in 20:4, whereas in the heart 18:2 n-6 is increased. This is possibly due to the relatively high rate of uptake of the fatty acids by the cardiomiocytes. Notably, docosahexaenoic acid decreases although its immediate precursor, 22:5 n-3, increases in the heart and in the liver. This cannot be explained on the basis of data available in the literature,[15] but one can speculate that, since the delta-4-desaturation activity is involved in both the metabolic pathways of the n-3 and n-6 series,[11,15] it may be differently affected by precursors of the two pathways. Finally, our data appear in contrast with earlier observations concerning the rapid uptake of dietary n-3 PUFA by tissues and membranes. This is related to the different intake of fatty acids: in our experimental conditions the diets contained the precursors of long-chain polyenoic fatty acids (LCP), whereas in the cited studies the diets directly contained LCP. Moreover, the period of dietary treatment was longer in comparison to literature protocols and this may suggest that in the long period the membrane fatty acid pattern tends to an

Table 2 Fatty acid composition (mol %) of liver plasma membrane

Fatty acid	O	OBC	BC
16:0	22.20 ± 1.77	24.91 ± 0.74	24.02 ± 3.37
16:1	2.51 ± 1.09	2.31 ± 0.36	3.20 ± 1.09
18:0	14.50 ± 2.71	14.44 ± 1.31	13.23 ± 1.26
18:1	20.02 ± 4.27	15.40 ± 2.78[c]	10.53 ± 0.76[ad]
18:2 n-6	14.13 ± 3.62	12.26 ± 0.48[b]	13.63 ± 5.17
20:3 n-6	0.27 ± 0.14	0.58 ± 0.16	0.95 ± 0.21[ad]
20:4 n-6	14.13 ± 3.62	17.26 ± 0.48[c]	18.26 ± 1.58[c]
20:5 n-3	0.67 ± 0.15	0.42 ± 0.28[c]	1.06 ± 0.42[ce]
22:4 n-6	2.26 ± 0.82	2.42 ± 0.83	1.93 ± 0.71
22:5 n-6	1.07 ± 0.67	0.35 ± 0.18[c]	1.02 ± 0.23[d]
22:5 n-3	n.d.	0.44 ± 0.15[a]	0.91 ± 0.47[ae]
22:6 n-3	6.80 ± 2.48	5.51 ± 0.93	6.24 ± 1.27
U.I.	166.66	161.79	176.99

The values are means ± SD of two determinations of eight rats per group.
[a]Significantly different ($P < 0.001$) compared with olive oil group.
[b]Significantly different ($P < 0.005$) compared with olive oil group.
[c]Significantly different ($P < 0.05$) compared with olive oil group.
[d]Significantly different ($P < 0.001$) compared with olive–black currant oil mixture group.
[e]Significantly different ($P < 0.005$) compared with olive–black currant oil mixture group.
[f]Significantly different ($P < 0.05$) compared with olive–black currant oil mixture group.

Table 3 Fatty acid composition of plasma membrane of heart

Fatty acid	O	OBC	BC
16:0	17.88 ± 2.83	17.02 ± 0.84	17.60 ± 3.43
16:1	1.40 ± 0.52	1.16 ± 0.56	1.86 ± 0.33
18:0	18.95 ± 0.80	18.01 ± 3.70	18.40 ± 1.14
18:1	14.51 ± 3.25	14.39 ± 5.28	10.57 ± 2.65[c]
18:2 n-6	9.57 ± 1.77	14.26 ± 1.20[a]	14.76 ± 5.32[c]
20:3 n-6	0.18 ± 0.10	0.39 ± 0.08[a]	0.41 ± 0.11[a]
20:4 n-6	17.68 ± 2.00	17.30 ± 2.83	16.72 ± 1.52
20:5 n-3	0.20 ± 0.04	0.58 ± 0.19[a]	0.51 ± 0.19[a]
22:4 n-6	2.78 ± 0.93	2.41 ± 0.44	2.51 ± 1.19
22:5 n-6	0.99 ± 0.65	0.36 ± 0.01[c]	0.60 ± 0.19[e]
22:5 n-3	n.d.	1.02 ± 0.31[a]	1.12 ± 0.46[a]
22:6 n-3	10.99 ± 1.86	9.37 ± 2.04	8.24 ± 0.94[b]
U.I.	189.32	190.10	180.69

The values are means ± SD of two determinations of eight rats per group.
[a]Significantly different ($P < 0.001$) compared with olive oil group.
[b]Significantly different ($P < 0.005$) compared with olive oil group.
[c]Significantly different ($P < 0.05$) compared with olive oil group.
[e]Significantly different ($P < 0.005$) compared with olive–black currant oil mixture group.

Table 4 Fatty acid composition of plasma membrane of brain

Fatty acid	O	OBC	BC
16:0	16.00 ± 0.92	16.27 ± 0.59	16.27 ± 0.61
16:1	0.60 ± 0.09	0.68 ± 0.18	0.75 ± 0.11
18:0	19.98 ± 0.41	19.87 ± 1.74	20.79 ± 1.02
18:1	25.19 ± 1.54	24.58 ± 2.01	23.92 ± 0.87
18:2 n-6	0.66 ± 0.07	0.96 ± 0.09[a]	0.93 ± 0.26[b]
20:0	0.66 ± 0.11	0.59 ± 0.08	0.69 ± 0.16
20:1	4.48 ± 0.33	3.95 ± 0.65	4.10 ± 0.53
20:3 n-6	0.20 ± 0.17	0.18 ± 0.03	0.28 ± 0.07
20:4 n-6	8.15 ± 0.30	8.18 ± 0.65	8.36 ± 0.28
20:5 n-3	0.50 ± 0.21	0.25 ± 0.17[b]	0.49 ± 0.23[c]
22:4 n-6	3.43 ± 0.49	3.92 ± 0.35	3.80 ± 0.32
22:5 n-6	5.81 ± 1.43	7.77 ± 0.34	5.49 ± 0.71
22:5 n-3	n.d.	n.d.	n.d.
22:6 n-3	9.08 ± 1.15	9.07 ± 0.64	9.31 ± 0.72
U.I.	164.61	164.59	165.77

The values are means ± SD of two determinations of eight rats per group.
[a]Significantly different ($P < 0.001$) compared with olive oil group.
[b]Significantly different ($P < 0.05$) compared with olive oil group.
[c]Significantly different ($P < 0.05$) compared with olive–black currant oil group mixture.

optimum that can be reached through the activation of compensatory mechanisms in the body (or tissue) to reverse the early effect of dietary unbalanced lipids.

Effect of dietary olive and black currant oils on brain plasma membrane fatty acid profile

Table 4 shows the fatty acid composition of plasma membrane isolated from brain of rats fed the experimental diets. It is evident that the fatty acid level remains substantially constant for all the diets used. It has to be noticed that high concentrations of 22:6 n-3, 20:4 n-6, and 22:4 n-6 were found in the brain cell membranes according to the literature.[25]

These LCPs of n-3 and n-6 families are derived by desaturation and elongation of the dietary essential fatty acids 18:3 n-3 and 18:2 n-6, respectively.[27] Then, in the experimental model tested, the administration of dietary BC oil, containing relatively high concentrations of the two LCP precursors compared with O oil, did not result in any appreciable difference in LCP content of brain membranes of the two groups of rats. This may apparently be in contrast to the results of other authors having shown significant changes in 20:5 n-3 and 4 n-6 in brain lipids of piglets[28] or rats[26] fed diets containing fish oils characterized by high LCP levels. However, the oils supplemented in our diets contained different amounts of LCP precursors, not LCP themselves. Thus, the different results obtained suggest that only the supplementation of high LCP-containing diets can alter the brain acyl group composition of membranes, which may even be harmful to animals (i.e., vitamin E deficiency

symptoms),[25] whereas diets based on the oils used by us do not change brain fatty acid composition, so risks of the above adverse effects are avoided. Finally, it has to be mentioned that the value for 22:5 n-6 with all the three diets used is similar to that reported by Galli et al[29] but higher than the value reported by Bourre et al.[25] This might be because we analyzed the composition of a plasma membrane-enriched fraction of the brain, while Bourre et al. analyzed the lipids extracted from the total forebrain homogenate. Moreover, since we carried out our experiments using adult rats, as compared with Bourre et al. who were investigating young rats, an age-related effect might be considered. However, recently Arbuckle et al.[30] reported values similar to ours for 22:5 n-6 of piglet synaptic plasma membrane phospholipids.

Effect of dietary olive and black currant oils on 5'-nucleotidase activity of plasma membranes

The activity of 5'-nucleotidase of plasma membrane-enriched fractions from liver, brain, and heart of rats fed various diets is shown in *Table 5*. There was no significant difference among the various dietary groups in terms of 5'-nucleotidase-specific activity either in heart or brain preparations, whereas a significant difference was found in the enzyme activity in liver membranes. In particular 5'-nucleotidase showed the highest catalytic activity in preparations from liver of rats fed O oil diet (37.16 U/mg), while no significant difference resulted between the enzyme activity of liver from rats fed either BC oil or OBC oil mixture. The enzyme activity of the control (sample taken before starting the experiments on membrane-enriched preparations from the three tissues of a group of rats) were not significantly different from those shown in *Table 5*, except that of membrane preparations from liver of rats fed the O oil diet.

We now analyze our data on the basis of various hypotheses reported on the effects of lipids on the activity of 5'-nucleotidase. Stanley and Luzio[31] maintain that the enzymatic activity is independent of membrane lipid composition, whereas Bernsohn and Spitz,[32] Zuniga et al.,[33] and Alam and Alam[34] maintain that dietary lipids are able to influence both membrane composition and enzymatic activity. In a previous trial on brain 5'-nucleotidase we have suggested an influence of metabolic changes involving the eicosanoids synthesis more than a direct effect of dietary lipids on the enzyme activity through the changes of the membrane fatty acid composition.[35]

In the experimental conditions used in our present study, the variations of 5'-nucleotidase activity do not seem again related to the modifications of membrane fatty acids (since the compositive peculiarity of the tested dietary lipids and for the small differences of unsaturation index do not allow one to propose changes in membrane physical properties such as fluidity). We believe that more likely that the high activity of 5'-nucleotidase in the liver plasma membrane of rats fed O oil could be related to eicosanoids synthesis. In fact, particularly in the aged rat liver, both the availability of prostaglandin precursors and the capacity of synthesizing prostaglandins decrease.[36,37] Therefore, a deficiency of dietary PUFA such as C18:3 n-6 and C18:4 n-3 could alter eicosanoid synthesis since the need of the above derivate fatty acids for a balanced synthesis of eicosanoids has been shown.[38] The altered synthesis of eicosanoids might influence the balance of vasocostrictor-vasodilator factors. 5'-nucleotidase could be involved, since its catalysis product is adenosine, a well-known mediator of arterial vasodilation.

Conclusions

Dietary oils differing mainly in the oleic acid, linoleic acid, and in 18C-PUFA content result in different fatty acid patterns of membrane-enriched fraction from the liver and to a lesser extent from the heart and the brain of experimental adult rats. The presence or not of 18C-PUFA (endogenously a product of delta-6-desaturase) in the diet does not result in significantly different patterns of their derivatives in the cell plasma membranes of any of the tissues examined. As expected, the major differences of the fatty acid patterns within the various organs examined have been found in the liver due to its intense metabolism of the dietary lipids.[39] Similarly we can explain the increase in 20:4 n-6 in the liver membranes of the BC oil diet groups, whereas the increase of 18:2 n-6 in the heart membranes could be the consequence of the relatively high rate of fatty acid uptake of the cardiomiocytes.[1] Also, since PUFA n-3 and PUFA n-6 compete for the same desaturases and elongases,[15,40] we find a slight decrease of the 22:6 n-3 with the BC oil diets as compared with the O oil diet. Moreover, when the products of the delta-6-desaturase are present in the diet (BC and BC-O mixture), levels of 22:6 n-3 do not increase, although its immediate precursor 22:5 n-3 increases in the liver and heart. This might be due to the decreased rate of 22:5 n-3

Table 5 5'-nucleotidase activity of plasma membrane of rat tissues

Tissue	O	OBC	BC
	nmol/min/mg		
liver	37.16 ± 7.76	22.10 ± 8.10[a]	27.57 ± 10.12[b]
heart	13.01 ± 3.45	14.70 ± 1.52	14.71 ± 5.25
brain	31.99 ± 5.40	27.40 ± 9.35	29.05 ± 9.23

The values are means ± SD of two determinations of eight rats per group.
[a]Significantly different ($P < 0.005$) compared with olive oil group.
[b]Significantly different ($P < 0.05$) compared with olive oil group.

desaturation consequent to possible regulation by precursors.

The activity of 5′-nucleotidase, a typical membrane-bound enzyme does not seem very sensitive to the diets used. However, it has to be noted that in the plasma membranes of liver from rats treated with the olive oil diet, the content of arachidonic acid is relatively low and the 5′-nucleotidase activity results are significantly higher than those found when rats were fed BC oil–based diets. This observation suggests that manipulation of the lipid environment influences 5′-nucleotidase activity either by the interaction of the enzyme with a specific membrane lipid or through complex lipid metabolic changes possibly involving eicosanoids synthesis and physiological effects. The present results will be the basis for investigation on the effects of diets rich in BC oil on the membrane composition, physical properties, and biochemical aspects of aged animals.

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