Dietary lipid effects on microsome fatty acid composition of liver and brain, on liver glucose-6-phosphatase, and on brain 5′-nucleotidase activity in the rat

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The effects of incorporation of dietary oils with different n6/n3 ratio and polyunsaturated fatty acids content into rat liver and brain microsomes has been studied. The investigation of membrane fatty acid composition of liver microsomes and that of brain microsomes gave different results. In particular, liver microsomes of rats fed fish oil showed a relatively higher content of 20:5n3 and 22:6n3, and a lower content of 20:4n6. Under these conditions, a reduced glucose-6-phosphatase activity was measured. Brain microsomal fatty acid composition was only slightly affected by dietary lipid intake. The 5′-nucleotidase activity of those particles was similar, although statistically different values were found in fish-oil–fed rats and in olive-oil–fed rats. The effects of membrane fatty acid composition on membrane-bound enzyme activity are discussed.

Keywords: dietary lipid; microsomal fatty acids; glucose-6-phosphate; 5′-nucleotidase

Introduction

Diet is an important factor for health and functional efficiency; it influences living organisms at different levels, including enzymatic activities.[1,2] There is considerable evidence that dietary fatty acid composition can influence adipose tissue composition and some aspects of intermediate metabolism (i.e., polyunsaturated fatty acids (PUFA) metabolism, regulation of lipogenesis and prostaglandin synthesis).[3-6]

Composition and functionality of cellular structures are also dependent upon dietary lipids.[7-10] For instance, the increase of polyunsaturated fatty acids in the phospholipids of biological membranes induces increased membrane fluidity, compressibility, and permeability.[11-12] Another aspect connected with lipid intake is the effect on the activity of numerous enzymes.[12-17] The dietary lipid effect on membrane-bound enzyme activity could be based on metabolic variations or membrane structural changes.

This study evaluates the effect of qualitatively different lipid dietary intakes on fatty acid composition of liver and brain microsomes, the activity of liver glucose-6-phosphatase (EC 3.1.3.9), and brain 5-nucleotidase (EC 3.1.3.5, 5′-ribonucleotide phosphohydrolase) in the rat. The two enzymes are active in different metabolic pathways, but are both membrane-bound and have been shown to depend on the chemical composition and physical state of the phospholipid environment in which they are embedded.[12,18,19] The analysis of compositive and functional data might provide unexplored information on the relation among dietary lipids, cellular structure composition, and enzyme activity.

The mechanisms whereby dietary lipid composition impacts on membrane composition include the competition of the n6 and n3 fatty acids for desaturation enzymes[3] and the inhibitory effect by PUFA on lipidosynthesis.[4] On this basis, the effect of different 18:2n6/18:3n3 ratios and PUFA content oils has been investigated. The final aim is to individuate which dietary

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intakes are able to determine variations not only in composition but also in functionality of structures, in order to improve lipid intake.

Materials and methods

Animals and diets

Experiments were performed on 24 Wistar rats that were 200±20g in weight. They were divided into 4 groups of 6 rats each and housed in cages at constant temperature (21 to 22°C) and relative humidity (55 to 56%).

The animals had food and water ad libitum. Diets were as follows:

Group A: fat-free diet + 10% grape-stone oil; Group B: fat-free diet + 10% olive oil; Group C: fat-free diet + 10% soybean oil; and Group D: fat-free diet + 10% fish oil. Fish oil was supplemented with 100 mg α-tocopherol per 100 g oil. The fish-oil–enriched diet was prepared every week and was stored at −20°C in sealed containers flushed with nitrogen.

Table 1 reports the fatty acid composition of the oils tested. The composition of 100 g of fat-free diet was: casein 30 g, sucrose and starch 64 g, salt mixture 4 g, cellulose 2 g, choline chloride and vitamins 0.1 g. After 3 months of experimental diets, the animals were sacrificed by cervical dislocation.

Preparation of microsomes

Organs were removed from sacrificed rats and weighed. Livers were suspended in 0.1 M tris/Cl solution and brains in 50 mM tris/Cl and 100 mM KCl solution pH 7.4. The homogenates were centrifuged for 20 minutes at 16,000*g*, and the resulting supernatants were centrifuged for 60 minutes at 105,000*g*. The resulting pellets were washed 3 times with respective buffers and resuspended in the same solutions.

All microsomal preparations were done at 0 to 4°C and kept frozen at −70°C until use. Microsomal protein concentrations were determined by the biuret method in the presence of 1% sodium cholate according to Bustamante et al.[20]

Table 1 Fatty acid composition (%) of the diets

Fatty acid	Grape-stone oil	Olive oil	Soybean oil	Fish oil
14:0				9.00
16:0	6.63	10.13	10.75	17.50
16:1		0.90		11.77
18:0	3.24	2.03	3.13	3.44
18:1	13.01	77.12	21.54	20.02
18:2n6	76.32	8.44	59.13	1.71
18:3n3	0.79	1.37	5.45	2.79
20:1				3.14
20:4n6				1.73
22:1				1.36
20:5n3				13.87
22:5n3				1.96
20:5n3				13.87
22:5n3				1.96
22:6n3				11.71
n6/n3	96.61	6.16	10.84	0.11

Enzyme assay

The 5′-nucleotidase activity was measured using 20 μg microsomal protein at 20°C in 2.5 ml of 50 mM tris/HCl, pH 7.4 containing 100 mM KCl and 20 μg of adenosine-deaminase (Sigma). The reaction was started by the addition of AMP (1 mM final concentration). The activity was determined spectrophotometrically by following the changes in extinction at 265 nm according to Ipata.[21] The glucose-6-phosphatase activity was evaluated as follows: in 1 ml 0.1 M sodium cacodylate buffer, pH 6.5, 70 μg microsomal protein and 20 mM glucose-6-phosphate were added; the mixture was incubated at 30°C for 10 minutes, then 4% trichloroacetic acid was added and centrifuged 5 minutes at 4000 rpm. 200 μl of supernatant was used for determining the released inorganic phosphate according to Marinetti.[22]

Fatty acid analysis

Lipids were extracted from microsomes according to Folch et al.[23] Fatty acids were methylated and composition analysis was performed on a Varian mod.3700 gas-chromatograph equipped with a glass column (2 m × 1.4 in 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.

Statistical analysis

All results are expressed as means ±SD. Statistical differences have been determined by a one-way analysis of variance and a Student Neuman Kuels test.[24]

Results

Fatty acid composition of liver microsomes of different groups (*Table 2*) reflects dietary lipid composition and PUFA metabolic trends. Group A shows high percentages of 18:2n6, 20:4n6, and 22:5n6, and low amounts of 22:6n3; group B shows a high percentage of 18:1n9, and balanced amounts of n3 and n6 derivatives; group C features considerable amounts of 18:2n6, 20:4n6, and 22:6n3; and group D shows remarkable percentages of 20:5n3 and 22:6n3, while fatty acids of the n6 serie appear in low amounts; n6/n3 ratio decreases from grape-stone to fish conditions; unsaturation index (U.I.) is slightly higher in groups A and D. Values of glucose-6-phosphatase (*Table 3*) show a significant decrease only in fish-oil–fed rats.

In different groups, the fatty acid composition of microsomes is more homogeneous in brain (*Table 4*)

Table 2 Liver microsome fatty acid composition (%)

Fatty acid	Grape-stone oil	Olive oil	Soybean oil	Fish oil
16:0	16.45 ± 0.08	16.39 ± 2.84	17.92 ± 1.28	19.65 ± 2.34[a,b]
16:1	1.46 ± 0.09	2.20 ± 0.24	1.99 ± 1.26	4.26 ± 1.71[a,b,c]
18:0	25.05 ± 1.60	27.50 ± 3.01	24.37 ± 1.53	21.36 ± 3.28[a,b]
18:1	9.24 ± 2.43	18.33 ± 1.98[a]	11.74 ± 1.26[a,b]	17.83 ± 1.84[a,c]
18:2n6	11.92 ± 0.83	3.21 ± 0.93[a]	13.63 ± 2.60[b]	2.14 ± 0.55[a,c]
20:4n6	23.21 ± 1.34	21.50 ± 0.69	17.56 ± 2.27[a,b]	4.99 ± 1.04[a,b,c]
20:5n3		tr	tr	9.99 ± 1.32
22:4n6	1.19 ± 0.11	0.85 ± 0.21	2.77 ± 1.26[a,b]	0.99 ± 0.52[c]
22:5n6	6.81 ± 1.66	2.46 ± 0.97[a]		1.66 ± 0.71[a]
22:5n3			0.69 ± 0.42	1.04 ± 0.26
22:6n3	1.52 ± 0.39	4.31 ± 0.41[a]	5.23 ± 0.66[a]	10.33 ± 2.12[a,b,c]
18:2n6/20:4n6	0.52	0.15	0.78	0.43
n6/n3	28.38	6.51	5.74	0.46
Unsaturation Index	175.31	154.51	157.14	175.72

Results are expressed as means ±SD. Values do not add to 100% because minor fatty acids are not reported. Statistical differences have been determined by a one-way analysis of variance and a Student Neuman Kuels test. If no superscript letter appears, values are not statistically different ($P > 0.05$). The superscript letters are referred to as follows: [a] significance with respect to grape-stone oil; [b] significance with respect to olive oil; [c] significance with respect to soybean oil.

than in liver. Nevertheless, different PUFA amounts are traced. These differences are connected with dietary intakes. Group A is particularly characterized by high percentages of n6 derivatives and a low amount of 22:6n3, connected with high dietary intake of 18:2n6 which limits n3 derivatives; in groups B and C, there are both n6 and n3 derivatives; group D, characterized by high dietary intakes of n3 serie, also shows a higher amount of these fatty acids in brain microsomes. Thus, the above compositive differences determine an unsaturation index similar in groups B, C, and D, and lower in group A.

The 5′-nucleotidase activity of the brain microsomes isolated from the four groups does not change remarkably as does the liver microsomal glucose-6-phosphatase. *Table 5* shows that the only significant change of the 5′-nucleotidase activity exists between the brain microsomes of fish-oil and olive-oil–treated rats.

Table 3 Glucose-6-phosphatase activity of rat liver microsomes

Diet	Glucose-6-phosphatase μmol/h/mg
Grape-stone oil	8.82 ± 1.30
Olive oil	9.36 ± 1.15
Soybean oil	7.41 ± 1.05[b]
Fish oil	4.03 ± 0.61[a,b,c]

Results are expressed as mean ±SD. Statistical differences have been determined by a one-way analysis of variance and a Student Neuman Kuels test. If no superscript letter appears, values are not statistically different ($P > 0.05$). The superscript letters are referred to as follows: [a] significance with respect to grape-stone oil; [b] significance with respect to olive oil; [c] significance with respect to soybean oil.

Discussion

The modification of the fatty acid composition of liver microsomes and, to a lesser extent, of brain microsomes was significant using the four different diets tested. Fatty acid compositions of membrane structures are the result of both direct dietary lipid incorporation and incorporation in phospholipids following metabolic changes determined by dietary intakes.

In order to interpret lower values of glucose-6-phosphatase activity in microsomes of liver from rats fed fish oil, we shall suppose different possibilities of lipid influence on the enzyme activity. Dietary lipids can affect chemical and physical properties of membranes; fatty acid composition changes of membrane phospholipids can be related to changes of either their packing or bilayer fluidity, or both.[12] Moreover, activities of membrane-bound enzymes can be modulated by particular phospholipids and/or by changes of membrane fatty acid composition.[25] On the other hand, enzyme activities may change also as a consequence of changes of metabolic pathway rates.[3,4,26]

If the structural possibility is considered, one can see that the fatty acid unsaturation index appears not to be directly related either with the activity of liver microsomal glucose-6-phosphatase, or with the activity of brain microsomal 5′-nucleotidase. When the activity of glucose-6-phosphatase in rats fed fish oil is compared to that of rats fed other oils, the peculiar composition of liver microsomes has to be considered as playing a significant role. In fact, only in rats fed fish oil have low percentage of 20:4n6 and relatively high percentage of 20:5n3 and 22:6n3 been found.

According to Bernsohn and Spitz,[18] the inhibitory effect on glucose-6-phosphatase could be related to an allosteric interaction of the enzyme with some n3 fatty acid metabolite. On the other hand, if we analyze the effect of fish oil diet on the enzyme activity from a

Table 4 Brain microsomes fatty acid composition (%)

Fatty acid	Grape-stone oil	Olive oil	Soybean oil	Fish oil
16:0	31.33 ± 1.75	27.09 ± 2.85[a]	28.46 ± 1.82[a]	26.26 ± 0.69[a]
16:1	3.76 ± 0.47	2.44 ± 0.25[a]	2.10 ± 0.19[a]	2.41 ± 1.11[a]
18:0	18.66 ± 1.25	19.80 ± 1.06	19.84 ± 0.97	19.92 ± 0.23
18:1	15.63 ± 4.40	18.02 ± 1.25	15.08 ± 3.68	16.96 ± 1.28
18:2n6	2.71 ± 0.97	1.07 ± 0.04[a]	2.14 ± 0.21[b]	0.23 ± 0.17[a,b,c]
20:0	0.73 ± 0.29	0.57 ± 0.12	1.51 ± 0.17[a,b]	1.34 ± 0.44[a,b]
20:1	0.67 ± 0.01	1.14 ± 0.56	0.81 ± 0.09	0.49 ± 0.46
20:4n6	9.35 ± 1.08	10.39 ± 0.48	11.67 ± 1.41[a]	8.01 ± 0.53[a,b,c]
20:5n3				0.97 ± 0.23
22:4n6	3.67 ± 1.19	3.33 ± 0.32	1.27 ± 0.46[a,b]	2.59 ± 1.04[c]
22:5n6	2.53 ± 0.56	1.85 ± 0.26	1.55 ± 0.35[a]	0.71 ± 0.97[a,b]
22:5n3				
22:6n3	5.79 ± 1.36	10.64 ± 1.26[a]	11.17 ± 1.27[a]	13.73 ± 0.80[a,b,c]
18:2n6/20:4n6	0.29	0.10	0.18	0.03
n6/n3	3.15	1.56	1.49	0.78
Unsaturation Index	124.95	151.71	148.8	153.68

Results are expressed as means ±SD. Values do not add to 100% because minor fatty acids are not reported. Statistical differences have been determined by a one-way analysis of variance and a Student Neuman Kuels test. If no superscript letter appears, values are not statistically different ($P > 0.05$). The superscript letters are referred to as follows: [a] significance with respect to grape-stone oil; [b] significance with respect to olive oil; [c] significance with respect to soybean oil.

metabolic point of view, it can be considered in relation to the inhibitory effect of polyunsaturated fatty acids on lipidosynthesis[4,27]; this inhibition might increase the lipid degradation for energy production with consequent carbohydrate economy, reduced gluconeogenesis rate, and depressed glucose-6-phosphatase activity.

The fatty acid composition of brain microsomes appears to be only scarcely influenced by dietary lipid intakes. Moreover, in the different groups of examined rats, particularly significant differences of long chain PUFA percentages and changes of the n6/n3 ratios are observed. This suggests an influence of metabolic changes due to different lipid intakes more than to a direct diet effect.

Microsomal 5′-nucleotidase, a glycoprotein relatively concentrated in the plasma membrane of mammalian cells, but also present in the membranes of cytoplasmic organelles,[28,29] exhibits only a slight diet-related difference in activity. The reduced 5′-nucleotidase activity of fish-oil–fed rats might be related to a diet very low in n6/n3 value, which might indirectly (i.e., via a prostaglandin mechanism) affect the enzyme activity. Our data also show that small amounts of 18:3n3 of olive and grape-stone oils can preserve fully active 5′-nucleotidase, contradicting, in our experimental conditions, the earlier suggestion of Bernsohn and Spitz[18] on the possible reduction of this enzyme activity due to n3 fatty acid deficiency in the diet.

5′-Nucleotidase is an enzyme catalyzing a reaction not immediately related metabolically to dietary intakes. Therefore, a direct metabolic effect on its activity following different lipid intakes is unlikely.

Table 5 5′-Nucleotidase activity of rat brain microsomes

Diet	nmol/min/mg
Grape-stone oil	3.72 ± 0.34
Olive oil	4.12 ± 0.63
Soybean oil	3.62 ± 0.48
Fish oil	3.12 ± 0.64[b]

Results are expressed as means ±SD. Statistical differences have been determined by a one-way analysis of variance and a Student Neuman Kuels test. If no superscript letter appears, values are not statistically different ($P > 0.05$). The superscript [b] refers to the significance with respect to olive oil.

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