Effect of n-6 and n-3 polyunsaturated fatty acids ingestion on rat liver membrane-associated enzymes and fluidity

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The influences of diets having different fatty acid compositions on the fatty-acid content, desaturase activities, and membrane fluidity of rat liver microsomes have been analyzed. Weanling male rats (35–45 g) were fed a fat-free semisynthetic diet supplemented with 10% (by weight) marine fish oil (FO, 12.7% docosahexaenoic acid and 13.8% eicosapentaenoic acid), evening primrose oil (EPO, 7.8% γ-linolenic acid and 70.8% linoleic acid) or a mixture of 5% FO-5% EPO. After 12 weeks on the respective diets, animals fed higher proportions of (n-3) polyunsaturated fatty acids (FO group) consistently contained higher levels of 20:3(n-6), 20:5(n-3), 22:5(n-3), and 22:6(n-3), and lower levels of 18:2(n-6) and 20:4(n-6), than those of the EPO (a rich source of (n-6) polyunsaturated fatty acids) or the FO + EPO groups. Membrane fluidity, as estimated by the reciprocal of the order parameter S_{DPH}, was higher in the FO than in the EPO or the FO + EPO groups, and the n-6 fatty-acid desaturation system was markedly affected.

Keywords: marine fish oil; evening primrose oil; desaturase activities; membrane fluidity; liver; rats

Introduction

It is now very well known that the biogenesis of membrane lipids is affected by exogenous fatty acids derived from dietary lipids. Fatty acid composition, and also fluidity of mammalian cell membranes, is changed by dietary modifications, depending on their constitutive unsaturated and saturated fatty acid contents.[1–3]

The endoplasmic reticulum of hepatocytes plays a key role in some essential cellular activities. The desaturases are integral amphipathic enzymes assembled to the NADH-cyt b_5 electron transferring system[4] located in the endoplasmic reticulum membrane where they control polyunsaturated fatty acid biosynthesis.[5] Earlier studies have suggested that alterations in the physical state of rat liver microsomal membranes could be correlated with changes in the activities of acyl chain desaturases.[3,6] The effect of fluidity on the desaturating enzymes implicates the existence of a self-regulatory mechanism in the membrane.[7]

It is also interesting that some fatty acids, either substrates or products of desaturases/elongases, are related to several diseases. Thus, there is substantial evidence that n-6 polyunsaturated fatty acids, mainly linoleic acid [LA, 18:2(n-6)] and more effectively, its metabolites γ-linolenic [GLA, 18:3(n-6)] and arachidonic [AA, 20:4(n-6)] acids, are capable of lowering plasma cholesterol levels and the incidence of atherogenesis.[8,9] On the other hand, those polyunsaturated fatty acids from the n-3 family, mainly eicosapentaenoic [EPA, 20:5(n-3)] and docosahexaenoic [DHA, 22:6(n-3)] acids, are related to the low incidence of coronary heart disease[10] and the suppression of platelet aggregation.[11]

To better understand the relationship between physiological functions and fatty-acid composition of microsomal lipids, we fed rats fish oil (source of EPA and DHA), evening primrose oil (source of LA and GLA), or a combination of both, and examined the effects on the physical property of membrane fluidity, as well as the activities of Δ^9, Δ^6, and Δ^5 desaturases in liver microsomes.

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Materials and methods

Weanling male (Wistar) rats, weighing 35–45 g, were randomly divided into three groups and housed in groups of six. All animals had free access to food and water. The animals were maintained on a fat-free semisynthetic diet supplemented with 10% (by weight) marine fish oil (FO), 10% evening primrose oil (EPO) or 5% FO-5% EPO. To minimize oxidation, all diets were prepared twice weekly and stored at 4° C under an atmosphere of nitrogen until needed. Changes in composition were not detected during storage. The composition of the diets is shown in *Tables 1 and 2*. A reduction of LA, EPA, and DHA was the major consequence of the combination FO + EPO supplemented to the fat-free semisynthetic diet.

After 12 weeks of treatment with the experimental diets, the animals were sacrificed and the livers were removed, weighed, and homogenized in an ice-cold medium (10 mmol/L Hepes (pH 7.4), 20 mmol/L ethylene-bis(oxyethylenenitrilo)-tetraacetic acid and 5 mmol/L dithiothreitol) by using an Omni-mixer. Each homogenate was centrifuged at 15,000*g* for 20 min at 4° C. The supernatant was collected and centrifugation (15,000*g*) was repeated. The 15,000*g* supernatant was centrifuged at 105,000*g* for 60 min in a Sorvall Ultracentrifuge (Norwalk, CT, USA), model OTD 50B. The resulting microsomal pellets were immediately frozen in liquid N_2 and stored at −70° C until the assays.

Lipids were extracted from the microsomal fractions by the method of Folch et al.[12] For the determination of fatty-acid composition, the samples were saponified by heating for 5 min with 5 mL of 0.2 M sodium methylate and heated again at 80° C for 5 min with 6% (wt/vol) H_2SO_4 in anhydrous methanol. The fatty-acid methyl esters formed were eluted with hexane and analyzed with a gas chromatograph (Hewlett-Packard, Palo Alto, CA USA: model 5710 A) equipped with a 60 m × 0.25 mm i.d. fused silica capillary column with 0.25 μm Supelcowax 10 film. A carrier gas (N_2) head pressure of 4 kg/cm^2, with a linear velocity of 33 cm/sec, was used. The initial column temperature was 200° C, which was held for 10 min, then programmed from 200–260° C at 2° C/min. The injection and detector temperatures were 275° C. Peak areas were calculated by a chromatograph-linked integrator. Individual fatty-acid methyl esters were identified on isothermal runs by comparison of their retention times against those of standards. Quantification of fatty-acid methyl esters was achieved by internal standardization (tricosanoic methyl ester, 23:0) using peak-area integration. Fatty-acid methyl esters for which no standard was available were quantified using calibration tables of relative response ratios constructed according to carbon number (using gas chromatography-mass spectrometry [GC-MS]). GC-MS was done on a Konik KNK-2000 chromatograph interfaced directly to an AEJ MS30/70 VG mass spectrometer, using the electron impact mode. The ion source temperature was maintained at 200° C, multiplier voltage was 4.0 kV, emission current was 100 μA and electron energy was 70 eV. The data were processed with a VG 11/250 data system.

Table 1 Composition of experimental diet

Basal diet (g/100 g)	
Milk *caseine*[a]	20.8
Cornstarch	19.6
Glucose	37.0
Cellulose powder	5.3
Vitamins[b]	1.0
Minerals[c]	6.3
Test oil (g/100 g)	10.0

[a]Lipid deprived.
[b]Vitamins (in 1 kg diet): retinyl acetate, 19800 IU; cholecalciferol, 6000 IU; thiamine HCl, 20 mg; riboflavine, 15 mg; niacin, 70 mg; pyridoxine HCl, 10 mg; inositol, 150 mg; cyanocobalamin, 50 μg; dl-α-tocopherol acetate, 170 mg; phylloquinone, 40 mg; Ca-panthotenate, 100 mg; choline-HCl, 1.36 g; folic acid, 5 mg; p-aminobenzoic acid, 50 mg; biotin, 0.3 mg.
[c]Minerals (in 1 kg diet): P, 7.75 g; Ca, 10.0 g; K, 6.0 g; Na, 4.0 g; Mg, 1.0 g; Mn, 80 mg; Fe, 0.3 mg; Cu, 12.5 g; Zn, 45 mg; Co, 90 μg; I, 0.49 mg.

Table 2 Fatty acid composition of dietary lipids

Fatty acid, weight %	FO	EPO	FO + EPO
14:0	3.8	—	1.6
16:0	13.9	8.5	11.8
16:1(n-7)	15.1	0.1	7.9
18:0	0.9	2.3	3.1
18:1(n-9)	26.2	9.8	18.0
18:2(n-6)	5.1	70.8	37.9
18:3(n-6)*	0.2	7.8	4.0
18:4(n-3)	2.5	—	—
20:0	—	0.2	0.1
20:1(n-9)	2.4	—	1.2
20:3(n-6)	0.4	—	0.2
20:4(n-6)	0.6	—	0.3
20:5(n-3)	13.8	—	6.8
22:0	—	0.1	—
22:4(n-6)	0.3	—	0.1
22:6(n-3)	12.7	—	6.3
Other	2.1	0.4	0.7

*γ-linolenic acid (GLA).

Microsomal protein determinations were made routinely by the method of Lowry,[13] using bovine serum albumin as standard.

Steady-state fluorescence polarization measurements of microsomes were made by using a 1 cm light path and excitation-emission wavelengths of 360–440 nm, in a Perkin-Elmer (Norwalk, CT USA) 650-40 spectrofluorometer as previously described[14] with slight modifications. The hydrophobic, fluorescence probe, 1,6-diphenyl-1,3,5-hexatriene (DPH), was used to label microsomal membranes. DPH was stored at −30° C in tetrahydrofuran at a concentration of 0.1 mg/mL. Native membranes, in an amount corresponding to about 200 μg of lipid, were incubated at 25° C for 1 hr with 1.55 μL DPH dispersed in 3 mL buffer saline (100 mmol/L KCl, 20% (wt/vol) sucrose, 50 mM Tris-HCl, pH 7.4). The "static component" of membrane fluidity was assessed by an order parameter, S, as described.[15]

Δ^9, Δ^6, and Δ^5 Desaturase microsomal activities were assayed as previously described[16] with modifications. The assay was initiated by adding 2–2.5 mg of microsomal protein to the incubation mixture, which contained 1.25 mmol/L NADH, 1.5 mmol/L reduced glutathione, 5 mmol/L ATP, 5 mmol/L $MgCl_2$, 0.3 mmol/L coenzyme A and 75 μmol/L ^{14}C-palmitic or 75 μmol/L ^{14}C-linoleic or 75 μmol/L ^{14}C-eicosa-8,11,14-trienoic acid in a final volume of 1 mL. Fatty acids from the n-6 family were used as sodium salt-albumin-complex (free

fatty acid:bovine serum albumin, 20:1 molar ratio). Incubations were carried out at 37° C for 20 min in a metabolic shaker. Enzyme assays were terminated by addition of 1 mL of 10% (wt/vol) KOH in ethanol followed by saponification at 100° C for 30 min and addition of 1 mL of 20% (wt/vol) H_2SO_4. Lipids were extracted twice with 5 mL chloroform: methanol (2:1, vol/vol). Both organic phases were combined and evaporated under nitrogen. Methylation of fatty acids was carried out with 14% boron trifluoride in methanol. Fatty acid methyl esters were separated on silica gel G-60 thin-layer chromatography (TLC) plates containing 5% (wt/vol) $AgNO_3$. Plates were developed three times at 4° C in benzene, and radioactivity was analyzed in a Berthold Automatic TLC-linear analyzer with the aid of a computer program.

Student's *t* test was used for the calculation of statistical significance.

Results and discussion

The results of our studies with rat-liver microsomes clearly show that different dietary fatty acids considerably altered fatty acid composition, fluidity, and acyl chain desaturase activities of the membrane.

Table 3 shows body weight gain, food intake, and liver weight data of the three experimental groups. A significant decrease was observed in the body and liver weights of the FO-fed group only. This profile cannot be due to differences in energy intake, but is consistent with the limitation in lipid accumulation in rat adipocytes attributed to the n-3 type of polyunsaturated fatty acids in the diet.[17]

The data in *Table 4* show the fatty-acid profiles of the liver microsomal lipids from rats fed FO, EPO, or FO + EPO diets. A significant reduction in LA and AA as well as an increase in eicosenoic acid (20:1(n-9)), dihomo-γ-linolenic acid (DGLA, 20:3(n-6)), EPA, docosapentaenoic acid (22:5(n-3)), and DHA were found in liver microsomes of the FO-fed rats. This is in agreement with findings reported on heart mitochondria in rats fed sardine oil,[18] and with a decreased Δ^5 desaturase activity *(Table 5),* as evident in the reduced proportion of AA to DGLA *(Table 4).*

Dietary changes did not have the same effects on the Δ^6 as on the Δ^5 desaturation of n-6 substrates *(Table 5).* One example of this is found in the EPO fed animals. This dietary treatment (a rich source of LA) involves a decrease of the Δ^6 desaturase activity. Because the Δ^6 desaturase is likely to be the main regulatory enzyme in the biosynthesis of AA,[19] the significant accumulation of LA in liver microsomes of EPO fed rats, in spite of the tendency toward decreased microsomal Δ^6 desaturation, might be indicative of a disturbed regulation of the biosynthesis of PUFA. Consistent with these results, Garg et al.[20] have recently shown a decrease in Δ^6 desaturase activity in hepatic microsomes after feeding of diets containing high amounts of LA. It is possible that the low "in vitro" activity may be attributable to dilution of the specific activity of [1-^{14}C]LA by endogenous LA pools.

Previous evidence has shown that DHA inhibits the Δ^6 desaturation of n-6 fatty acids.[20] In our experiments, the low levels of LA and AA in the FO or FO + EPO groups, despite the high intake of DHA, suggest a possible inhibitory effect of EPA on the conversion of DGLA to AA[21]; in agreement with an accumulation of DGLA and 22:5(n-3), as metabolite of EPA, in membrane microsomes of FO group. EPA, like AA, is readily incorporated into phospholipids,[20,22] possibly by competing for the incorporation sites as well as for cyclo-oxygenase and lipoxygenase systems.[23]

On the other hand, Δ^9 desaturase activity was lower in the EPO-fed group *(Table 5).* This was probably due to the high dietary content of LA, which tends to decrease this activity.[24]

The change in the type of membrane lipid unsaturation in rat liver microsomes clearly affects the mo-

Table 3 Influence of dietary lipids on body weight, food intake, and liver weight in rats

		FO	EPO	FO + EPO
Body wt (g)				
	start	38.2 ± 1.1	35.2 ± 3.2	40.1 ± 3.3
	final	215.4 ± 7.0	255.8 ± 3.3*	243.1 ± 3.4*
Food intake (g/day)		15.3 ± 1.3	16.3 ± 1.2	16.8 ± 0.9
Liver wt (g)		8.5 ± 0.2	10.4 ± 0.1*	11.1 ± 0.3*

Mean ± SEM of six rats per group.
*$P < 0.05$ versus FO, EPO, or FO + EPO groups.

Table 4 Fatty acid composition of liver microsomes in rats fed different dietary lipids

	FO	EPO	FO + EPO
12:0	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1
14:0	0.8 ± 0.2	0.9 ± 0.2	0.9 ± 0.1
16:0	22.3 ± 0.1	17.3 ± 0.4*	19.6 ± 0.4*
16:1(n-7)(n-9)	2.5 ± 0.3	0.3 ± 0.1*	0.8 ± 0.2*
17:0	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.2
18:0	16.6 ± 2.2	20.3 ± 1.4	17.5 ± 1.3
18:1(n-9)	4.8 ± 2.2	7.4 ± 1.2	6.8 ± 0.4
18:2(n-6)	2.7 ± 0.3	10.4 ± 0.3*	9.0 ± 0.4*
20:0	0.3 ± 0.1	0.4 ± 0.1	0.3 ± 0.1
20:1(n-9)	6.1 ± 0.6	3.0 ± 0.4*	5.2 ± 0.2
20:3(n-6)	2.4 ± 0.2	1.0 ± 0.3*	1.4 ± 0.2*
20:4(n-6)	8.5 ± 1.3	29.2 ± 2.3*	17.7 ± 5.1*
20:5(n-3)	9.4 ± 3.2	—	2.9 ± 1.4*
22:5(n-3)	3.0 ± 0.6	1.0 ± 0.1*	1.6 ± 0.2*
22:6(n-3)	15.3 ± 1.6	3.6 ± 0.5*	10.2 ± 2.2*
Other	4.7 ± 0.3	4.7 ± 0.7	5.9 ± 0.2
20:4(n-6)/20:3(n-6)	3.54	29.24*	12.64*
20:4(n-6)/18:2(n-6)	3.15	2.81*	1.97*
SFA[a]/PUFA	1.01	0.79*	0.89*
DBI[b]	211.4	177.9*	181.9*
DBI/SFA[c]	5.21	4.51*	4.69*

Mean ± SEM of six rats per group. The values are expressed as weight %.
[a]Saturated fatty acids.
[b]Double bond index.
[c]Unsaturation index.
*$P < 0.05$ versus FO, EPO, or FO + EPO groups.

Table 5 In vitro desaturase activities of liver microsomes of rats fed different dietary lipids

	FO	EPO	FO + EPO
Δ^9 [16:0→16:1(n-9)]	120.2 ± 3.3	74.8 ± 2.9*	84.2 ± 3.1*
Δ^6 [18:2(n-6)→18:3(n-6)]	140.1 ± 4.1	85.6 ± 3.2*	97.7 ± 2.8*
Δ^5 [20:3(n-6)→20:4(n-6)]	106.8 ± 3.6	150.4 ± 4.5*	132.1 ± 4.2*

Mean ± SEM of six rats per group. The activities are expressed as pmol/mg protein per min.
*$P < 0.05$ versus FO, EPO, or FO + EPO groups.

Table 6 Fluorescence polarization studies of liver microsomal membranes of rats fed different dietary lipids

	FO	EPO	FO + EPO
Fluorescence anisotropy (r)	0.180 ± 0.002	0.190 ± 0.002*	0.187 ± 0.001*
Anisotropy parameter $[(r^o/r)-1]^{-1}$	0.973 ± 0.021	1.084 ± 0.016*	1.053 ± 0.012*
Order parameter (S)	0.619 ± 0.006	0.648 ± 0.005*	0.640 ± 0.004*

Mean ± SEM of six rats per group. Anisotropy estimations were at 25° C.
*$P < 0.05$ versus FO, EPO, or FO + EPO groups.

lecular ordering of the membrane lipids. In the steady-state fluorescence polarization studies carried out with microsomes of FO, EPO, and FO + EPO groups *(Table 6)*, the order parameter S_{DPH} is considered to be the reciprocal of the membrane fluidity. Therefore, microsomal membrane fluidity was enhanced by FO dietary treatment. Although the mechanism whereby such compositional changes affect the physical properties of the membrane lipids is unknown, it is evident that this might alter the bound enzyme system or the affinity for binding substrates and thus lead to the observed activities.

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