Biochimica et Biophysica Acta, 1086 (1991) 295–304

BBALIP 53785

Interactive effects of dietary ($n-3$) polyunsaturated fatty acids and chronic ethanol intoxication on synaptic membrane lipid composition and fluidity in rats

M. Zérouga [1], F. Beaugé [1], E. Niel [1], G. Durand [2] and J.M. Bourre [1]

[1] *INSERM U 26, Hôpital Fernand Widal, Paris (France) and* [2] *INRA, Jouy en Josas (France)*

(Received 11 April 1991)
(Revised manuscript received 18 July 1991)

Key words: $n-3$ Polyunsaturated fatty acid; Synaptic vesicle; Plasma membrane; Lipid composition; Membrane fluidity; Fluorescence polarization; (Rat brain)

The influence of dietary polyunsaturated fatty acids on fatty acid composition, cholesterol and phospholipid content as well as 'fluidity' (assessed by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) probes) of brain synaptic plasma membranes (SPM) and their interactions with chronic ethanol effects were studied in rats fed for two generations with diets either devoid of ($n-3$) fatty acids (sunflower oil diet), rich in α-linolenic acid (soya oil diet) or in long chain ($n-3$) fatty acids (sunflower + cod liver oil diet). Results were compared with rats fed standard lab chow. Sunflower oil led to an increase in the $(n-6)/(n-3)$ ratio in the membranes with an increase of the 'fluidity' at membrane apolar level; sunflower + cod liver oil decreased the $(n-6)/(n-3)$ ratio without affecting membrane 'fluidity' while no difference was seen between the SPM of rats fed soya oil and standard diet. After 3 weeks alcohol intoxication in rat fed the standard diet: oleic α-linoleic acids and cholesterol levels were increased, arachidonic acid and the double bond index/saturated fatty acids were decreased and there was a decrease of 'fluidity' in the lipid core of the SPM. Soya oil almost totally abolished these usually observed changes in the SPM fatty acids composition but increased oleic acid and cholesterol without any change in fluidity. Sunflower oil led to the same general alterations of fatty acid as seen with standard diet but to a greater extent, with decrease of the 'fluidity" at the apolar level and in the region probed by TMA-DPH. When sunflower oil was supplemented with cod liver oil, oleic and α-linoleic acids were increased while the 'fluidity' of the apolar core of SPM was decreased. So, the small changes in fatty acid pattern seem able to modulate neural properties i.e. the responses to a neurotoxic like ethanol. A structurally specific role of PUFA is demonstrated by the pernicious effects of the α-linolenic acid deficient diet which are not totally prevented by the supply of long chain ($n-3$) PUFA.

Introduction

Although the fatty acid composition in brain lipids is known to be relatively resistant to dietary influence as compared to other organs, noticeable alterations in synaptic polyunsaturated fatty acid (PUFA) profiles were observed in rats fed diets deficient in α-linolenic acid (C18:3($n-3$)) [1]. Furthermore, there is now strong evidence that dietary lipids induced alterations in membrane composition influence brain-membrane-associated events [2], perhaps through disturbances in the physical properties of these membranes.

Abbreviations: S, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; C18:1($n-9$), oleic acid; C18:2($n-6$), linoleic acid; C18:3($n-3$), alpha linolenic acid; C20:3($n-6$), gamma linolenic acid; C20:4($n-6$), arachidonic acid; C22:5($n-6$), docosapentaenoic acid; C22:6($n-3$), docosahexaenoic acid; DBI, double bond index; Ch/PL, cholesterol/phospholipids molar ratio; SPM, synaptic plasma membranes; P, degree of fluorescence polarization; DPH, 1,6-diphenyl-1,3,5-hexatriene; TMA-DPH, 1-(4-trimethylammoniumphenyl)-6-phenylhexa-1,3,5-triene; PROP-DPH, 3-*p*(6-phenyl)-1,3,5-hexatrienylphenyl propionic acid.

Correspondence: J.M. Bourre, INSERM U26, Hôpital Fernand Widal, 200, rue du Fb. Saint Denis, 75010 Paris, France.

Alterations of membrane physicochemical properties are important primary effects of ethanol, a well-recognized neurotoxin [3]. Ingestion of ethanol has a variety of effects on membrane function and affects membrane 'fluidity' [4] which is an approach for the study of physical membrane properties. Previous studies have shown that ethanol intoxication alters membrane composition of fatty acids [5,6] as well as cholesterol content [7]. The most consistent finding has been, besides increases in oleic and in linoleic acids, a decrease in arachidonic acid together with decreases in other PUFA of the $(n-3)$ and $(n-6)$ series. Some of these changes may result from adaptative mechanisms in order to counteract the biophysical effects of ethanol [8]. It is therefore clear that there could be an interplay between the ingested diet and alcohol intoxication at the membrane level. The effects of ethanol may be modified by the availability of an exogeneous supply of PUFA. Changes in the fatty acid pattern may be sufficient to alter neural properties and thus affect tolerance to and dependence on ethanol. Furthermore, dietary and nutritional factors may have confounding effects on the responses to ethanol and could explain conflicting results concerning the changes in membrane lipid composition after ethanol intoxication [9,10].

The present study was thus undertaken in rats to elucidate the effects of various diets with different $(n-3)$ fatty acid contents and $(n-6)/(n-3)$ balances and their interaction with chronic ethanol intoxication. Synaptic membrane fatty acid composition as well as phospholipid and cholesterol concentrations were studied in parallel to the changes in membrane 'fluidity' as demonstrated by fluorescence polarization of different 1,6-diphenyl.1,3,5 hexatriene (DPH) probes which incorporate at different depths in the membrane bilayers [11–13].

Materials and Methods

Diets

Four semi-synthetic diets were used. They differed mainly by the amount or the nature of $(n-3)$ PUFA (Table I). The diets consisted of: (1) a sunflower oil diet containing 1.6 g/100 g of sunflower oil which is deficient in α-linolenic acid (C18:3$(n-3)$); (2) a soya oil diet containing 1.9 g/100 g of soya oil; and (3) a sunflower oil supplemented with cod liver oil (CLO), containing 1.7 g/100 g of sunflower and 0.3 g/100 g of CLO.

The soya oil and fish oil diets were rich in PUFA of the $(n-3)$ series in a proportion of about 1.5 mg/g, but they differed in their nature. The soya oil diet contained only α-linolenic acid whereas the sunflower oil + CLO diet contained eicosapentaenoic acid (C20:5$(n-3)$) and docosahexaenoic acid (C22:(6$n-$3)). All these three diets supplied equivalent amounts of linoleic acid (C18:2$(n-6)$) (approx. 10 mg/g of diet) which covered the requirement in essential $(n-6)$ PUFA [14,15], leading to a ratio $(n-6)/(n-3)$ of about 7 for soya oil and fish oil diets and 344 for the sunflower diet (Table II). These three diets have been elaborated and used by Dr. G. Durand and his group (INRA, Jouy en Josas) [16].

(4) A standard diet (Extra-Labo, Ets. Pietrement, France) containing 5 g/100 g of a mixture of oils (α-linolenic and linoleic acid 1.1 mg/g of diet and 15 mg/g of diet, respectively) with a $(n-6)/(n-3)$ ratio of about 14 (Table II) The diets were stored in the dark at 4°C and tested regularly for lipid peroxidation [17].

TABLE I

Composition of the experimental diet [a]

Ingredients (g/100g)	Diets		
	soya	sunflower	sunflower + cod liver
Starch	45.94	46.24	45.84
Sucrose	23.00	23.00	23.00
Casein	22.00	22.00	22.00
D,L-Methionine	0.16	0.16	0.16
Vitamin mixture [b]	1.00	1.00	1.00
Mineral mixture [c]	4.00	4.00	4.00
Cellulose	2.00	2.00	2.00
Lipids: Soybean oil	1.9		
Sunflower oil		1.6	1.3
Cod liver oil			0.7

[a] Standard diet contained the following nutrients per 100 g: 22 g of casein, 63.84 g of carbohydrates, 5 g of lipids, 0.16 g of DL-methionine, 1 g of vitamin mixture, 4 g of mineral mixture and 4 g of cellulose.

[b] Composition of vitamin supplement per kilo (Triturated in dextrose). United States Biochemical Corp., Cleveland (USA). α-Tocopherol (1000 I u/gm), 5.0 g; L-ascorbic acid, 45.0 g; choline chloride, 75.0 g; D-calcium pantothenate, 3.0 g; inositol, 5.0 g; menadione, 2.25 g; niacin, 4.5 g; para-aminobenzoic acid, 5.0 g; pyridoxine HCl, 1.0 g; riboflavin, 1.0 g; thiamin HCl, 1.0 g; vitamin A acetate, 900000 units; calciferol (vitamin D_2), 100000 units; biotin, 20 mg; folic acid, 90 mg; vitamin B_{12}, 1.35.

[c] Composition of the mineral mixture/100 g $CaHPO_4 \cdot 2\,H_2O$, 38.0; K_2HPO_4, 24.0; $CaCO_3$, 18.1; NaCl, 7.0; MgO, 2.0; $MgSO_4 \cdot 7\,H_2O$, 9.0; $FeSO_4 \cdot 7\,H_2O$, 0.7; $ZnSO_4 \cdot H_2O$, 0.5; $MnSO_4 \cdot H_2O$, 0.5; $CuSO_4 \cdot 5\,H_2O$, 0.1; NaF, 0.1; $Al_2(SO_4)_3$ $K_2SO_4 \cdot 24\,H_2O$, 0.02; KI, 0.008; $CoCO_3$, 0.008; $Na_2SeO_3 \cdot 5\,H_2O$, 0.001.

Animals

21-day-old Wistar rats weighing 40 to 50 g (IFFA CREDO, France) were fed with the semi-synthetic standard diet. Two weeks before mating, female rats were randomly divided into four groups. A first group was left on the same diet. The three other groups were given either sunflower oil, soya oil or sunflower + CLO. These females were then mated at 10 weeks of age. The males were Wistar rats receiving the standard diet.

TABLE II

Fatty acid composition of the diets

Values represent the average obtained from at least three separate extractions and analyses of the respective diets and are relative amounts, expressed as a percentage of the total identified fatty acids by weight. DBI, double bond index, see Table VI

Oil percentage in the diet (g/100g):	Soy-bean (1.9%)	Sun-flower (1.6%)	Sunflower + cod liver (2% 1.3% *Sf* 0.7% *CL*)	Stand-ard (5%)
Σ Saturated	15.7	11.6	13.5	33.7
C14:0	–	–	1.2	1.7
C16:0	11.0	6.6	7.3	20.7
C17:0	–	–	0.2	0.5
C18:0	3.7	4.0	3.5	10.3
C20:0	0.6	0.3	0.2	0.3
C22:0	0.4	0.7	0.5	0.2
C24:0	–	–	0.2	–
Σ Monounsaturated	23.4	19.4	29.3	33.6
C16:1($n-9$)	–	–	0.2	1.7
C16:1($n-7$)	–	0.2	2.6	–
C18:1($n-9$)	21.1	18.0	18.9	28.8
C18:1($n-7$)	1.9	1.0	2.1	2.7
C20:1($n-9$)	0.4	0.2	3.7	0.4
C22:1($n-11$)	–	–	1.8	–
Σ Polyunsaturated ($n-6$)	53.5	68.8	49.2	30.5
C18:2($n-6$)	53.5	68.8	49.0	30.3
C20:4($n-6$)	–	–	0.2	0.2
Σ Polyunsaturated ($n-3$)	7.4	0.2	8.0	2.2
C18:3($n-3$)	7.4	0.2	0.4	2.2
C18:4($n-3$)	–	–	0.8	–
C20:5($n-3$)	–	–	3.0	–
C22:5($n-3$)	–	–	0.3	–
C22:6($n-3$)	–	–	3.5	–
mg ($n-6$)/(100 g)	955.0	1034.0	924.0	1433.0
mg ($n-3$)/(100 g)	132.0	3.0	150.0	103.0
$n-6/n-3$	7.2	344.0	6.2	14.0
DBI/S	9.7	13.5	12.6	3.0

Female rats were maintained on their diet throughout pregnancy and during suckling. The litters weaned at 21 days received the same diet through another generation. The males from the second generation were used for this work. In the overall study, on post partum day four the litters were equalized to 9 pups each.

Chronic alcohol administration

At 7–8 weeks of age (210 to 230 g), the rats from each diet group were divided into 2 groups, alcohol treated group and pair-fed control group and housed individually. Ethanol (20% v/v in water) was administered daily to alcohol-treated rats by intragastric intubation for 21 days. The initial dose was 3 g/kg body weight (bwt) and was increased by 0.5 g/kg every 3 days [18]. The control rat group received a mixture of 50% starch and 50% sucrose solution by intubation at a dose equicaloric with that of ethanol and were pair-fed the same amount of the different experimental diets as the alcohol treated animals.

Preparation of synaptosomal membranes

The rats were killed by decapitation without anesthesia and the whole brains were rapidly dissected and individually homogenized with a glass homogenizer in 9 vol. of 0.32 M of sucrose containing 5 mM of Tris-HCl buffered at pH 7.4. The homogenate was used for synaptosomal membrane preparation by ultracentrifugation according to Çotman and Matthews [19] as previously described [4]. At the end of preparation, the synaptosomal membrane fraction was carefully rinsed from Ficoll by adding phosphate buffer 80 mM (pH 7.4) and centrifuged ($35\,000 \times g$) 3 times for 30 min. The final pellet was resuspended in phosphate buffer (pH 7.4) and stored at −80°C until analysis.

The protein concentration (1.5 to 2 mg/ml) was determined according to Lowry et al. [20]. The enzyme activity of the Na^+/K^+-ATPase was increased 4-fold as compared to the activity in the homogenate.

Lipid extraction

Thawed membrane suspensions were extracted with isopropanol and chloroform according to the method of Rose and Oklander [21]. To complete the extraction, the evaporated extract was transferred into chloroform/methanol (2/1) and washed with 0.2 volume of 0.1 M KCl. The cholesterol content was determined enzymatically [22] and the total phospholipid content by spectrophotometric assay for phosphorus after total mineralization according to Chen et al. [23].

The fatty acid composition of phospholipids was determined after hydrolysis and methylation according to Morisson and Smith [24]. The fatty acid methyl esters were analyzed with a Carlo Erba model gas liquid chromatograph equipped with automatic injector 'on column', a flame ionization detector and a C.P WAX 52 C.B. bonding fused silica capillary column (50 m × 0.3 mm internal diameter). The assays were carried out with a programmed oven temperature rise of 3°C/min from 54 to 220°C. The pressure of H_2 carrier gas was 0.8 Bar. Peak areas were measured with an integrator on-line with a microcomputer giving automatic expression of data.

Fluorescence probe polarization

Three fluorophores were used in this study: 1,6-diphenyl-1,3,5-hexatriene (DPH) for probing the membrane lipid deep core [11] or its polar derivatives; 1-(4-trimethylammonium-phenyl)-6 phenyl-1,3,5-hexatriene (TMA-DPH); and 3-*p*-(6-phenyl)-1,3,5-hexatrienyl phenyl propionic acid (PROP-DPH) for probing the polar region membrane [12,13]. A diluted aliquot containing about 15 μg protein/ml from the

thawed synaptosomal membrane fraction in phosphate buffer (pH 7.4) was incubated at 37°C either for 45 min with 1 μl of DPH, or for 30 min with TMA-DPH or PROP-DPH. The probes were initially dissolved in dimethyl sulfoxide. The final concentration for all probes in the incubating medium was 2 μM.

Steady state fluorescence polarization studies were performed at 25°C using a T-Format 'Spectrophotofluorimetre' (SEFAM, Nancy, France). The temperature in the cuvette was thoroughly maintained at 25.0 −/+ 0.1°C. The excitation wavelength was 360 nm, the emission being detected at 430 nm. The degree of fluorescence polarization P for each probe was calculated after Shinitzky et Barenholz [25].

$$P = \frac{I// - I^{\perp}}{I// + I^{\perp}}$$

Where $I//$ and $I^{\perp}$ are the polarized fluorescence intensities measured horizontally and perpendicularly to the polarized exciting light. After determination of the basal values and to study the acute effect of ethanol, 0.350 to 1.050 M (final concentration) were added in the cuvette and P determined again.

Statistical analysis

For comparison of the resulting values, membranes from control and ethanol treated animals which were prepared on the same day and analyzed at the same time were paired and the differences between these pairs were evaluated by Student's 't' test for paired samples.

Results

Animal postnatal growth

At weaning, the rats fed the sunflower oil diet had, significantly lower body weight (−20%) compared to rats fed the standard diet. There was no difference between the rats fed either soya oil or sunflower oil + CLO and those fed standard diet. From weaning to 7 weeks, the weight gain of the animals fed soya, sunflower or sunflower + CLO diet was slightly less than that of the animals fed standard diet, particularly with the sunflower diet (Table III). During the 3 weeks of ethanol treatment, the average weight gain was reduced (from 57% in the sunflower + CLO diet group to 70% in the standard diet group) partly in consequence of the pair-feeding of the control rats as a function of the amount of chow ingested by the ethanol treated rats. In addition, the weight gain was lower for the sunflower + CLO treated rats than for the pair-fed isocaloric group whereas no differences were seen in the other three groups (Table IV).

TABLE III

Effect of dietary fat on rat body growth

Values are means ±S.E. of results obtained from (n) animals. (n), number of animals

Diets	Body weight at weaning (3 weeks) (g)	Weight gain from 3 to 7 weeks (g/week)
Standard (25)	44.0 ± 1.0	44.5 ± 0.9
Soya (30)	45.0 ± 1.0	41.3 ± 1.2 [c]
Sunflower (32)	34.0 ± 0.9 [a]	37.0 ± 0.8 [a]
Sunflower + CLO (30)	44.0 ± 1.0	40.5 ± 1.0 [b]

[a] $P < 0.001$.
[b] $0.001 < P < 0.01$.
[c] $0.01 < P < 0.02$ compared to standard diet group.

Effect of dietary fat on lipid composition of synaptic membranes

The fatty acid composition of the synaptic membranes was found rather stable or very regulated. Even when the DBI/S ratio varies from 3 to 13.5 and the $(n-6)/(n-3)$ ratio from 6. to more than 300 in the different diets used in the present study, the same parameters especially the DBI/S (about 3.8) were similar in the SPM of rats fed those different diets and the $(n-6)/(n-3)$ ratio varied only from 0.7 to 2.7.

In comparison with the standard diet, no difference in fatty acid composition was found with rats fed the soya oil diet. When the animals were fed the sunflower oil diet, the proportion of C22:6($n-3$) was decreased

TABLE IV

Weight gain of rats fed different diets and intoxicated or not with ethanol for three weeks

See legend to Table III

Diets	Food intake (g/day)		Weight gain (g/week)	
	control	ethanol	control	ethanol
Standard.	12.0 ± 1.0 (9)	12.0 ± 1.0 (9)	13.8 ± 0.5 (9)	14.5 ± 0.5 (9)
Soya	11.0 ± 0.5 (6)	11.0 ± 0.5 (6)	16.5 ± 0.6 (6)	17.0 ± 0.6 (6)
Sunflower	12.0 ± 0.5 (6)	12.0 ± 0.5 (6)	14.8 ± 0.4 (6)	13.8 ± 0.3 (6)
Sunflower ± CLO	11.5 ± 0.6 (9)	11.0 ± 1.0 (9)	17.5 ± 0.6 (9)	14.2 ± 0.5 [a](9)

[a] $0.02 < P < 0.05$ compared to control group.

and that of C22:5($n-6$) increased, resulting in an increase of the ($n-6$)/($n-3$) molar ratio (2.7 versus 1.0 in SPM of rats fed standard diet), but the total amount of PUFA was not altered. The C18:1($n-9$) content was also significantly decreased. The total amount of the monounsaturated fatty acids (MUFA), of the saturated fatty acids (S) and of the double bond index/saturated fatty acids ratio (DBI/S) were not

TABLE V

Fatty acid composition of synaptic membranes from rats fed different diets and intoxicated or not with ethanol

Results are expressed as % of total fatty acids. Values are means ± S.E. from (n) animals

Diets:	Standard		Soya		Sunflower		Sunflower + CLO	
Treatment:	control (7)	ethanol (7)	control (5)	ethanol (5)	control (4)	ethanol (4)	control (7)	ethanol (7)
C14:0	0.20 (0.01)	0.60 (0.07) [b]	0.40 (0.02)	0.40 (0.02)	0.60 (0.05) [e]	2.7 (0.3) [a]	0.30 (0.03)	0.40 (0.03)
C16:0	21 (0.2)	22.4 (0.7) [b]	20.6 (1.0)	20.0 (0.2)	19.7 (0.8)	19.5 (0.1)	20.8 (0.1)	20.3 (0.4)
C16:1($n-9$)	1.00 (0.02)	0.90 (0.04)	0.80 (0.01)	0.80 (0.04)	0.70 (0.02)	0.80 (0.02)	0.70 (0.07)	1.0 (0.1)
C16:1($n-7$)	0.8 (0.1)	0.9 (0.1)	0.90 (0.01)	0.9 (0.1)	1.0 (0.1)	0.20 (0.00)	2.7 (0.7) [e]	1.9 (0.1)
C17:0	0.60 (0.02)	0.8 (0.1)	0.70 (0.02)	0.60 (0.03)	1.5 (0.1)	2.6 (0.2) [b]	0.40 (0.03)	0.4 (0.0)
C18:0	21.5 (0.2)	20.4 (0.2)	22.0 (0.6)	21.7 (0.6)	20.0 (0.7)	18.3 (0.2)	21.1 (0.2)	21.2 (0.2)
C18:1($n-9$)	15.8 (0.2)	16.4 (0.3) [d]	15.2 (0.1)	16.0 (0.7) [d]	13.6 (0.2) [e]	14.1 (0.1) [d]	16.6 (0.3)	17.8 (0.4) [b]
C18:1($n-7$)	4.0 (0.1)	3.9 (0.1)	4.2 (0.1)	4.0 (0.1)	4.4 (0.2)	4.7 (0.1)	3.9 (0.1)	4.0 (0.3)
C18:2($n-6$)	0.9 (0.1)	1.20 (0.04) [d]	0.80 (0.05)	0.9 (0.1)	0.60 (0.04) [e]	0.9 (0.1) [b]	0.80 (0.07)	1.1 (0.1) [c]
C20:0	0.40 (0.04)	0.50 (0.02)	0.40 (0.04)	0.40 (0.02)	1.1 (0.1) [f]	2.1 (0.1) [c]	0.40 (0.03)	0.40 (0.03)
C20:1($n-9$)	0.80 (0.02)	0.90 (0.07)	0.80 (0.04)	0.70 (0.02)	0.90 (0.05)	1.3 (0.1)	0.80 (0.03)	0.90 (0.03)
C20:1($n-7$)	0.30 (0.02)	0.30 (0.01)	0.30 (0.02)	0.30 (0.02)	0.40 (0.03)	1.2 [c] (0.1)	0.30 (0.03)	0.40 (0.01)
C20:2($n-6$)	–	–	0.30 (0.02)	0.20 (0.02)	0.20 (0.05)	0.40 (0.02)	0.20 (0.02)	0.20 (0.06)
C20:3($n-6$)	0.50 (0.04)	0.40 (0.04)	0.20 (0.02)	0.20 (0.02)	0.30 (0.01)	0.30 (0.01)	0.60 (0.03)	0.60 (0.03)
C20:4($n-6$)	9.8 (0.3)	9.0 (0.3) [d]	9.9 (0.1)	9.9 (0.3)	10.1 (0.1)	8.4 (0.1) [a]	8.4 (0.1) [f]	8.2 (0.4)
C22:0	0.40 (0.02)	0.60 (0.07)	0.40 (0.05)	0.40 (0.02)	0.30 (0.02)	0.70 (0.02) [d]	0.30 (0.03)	0.50 (0.03)
C22:1($n-9$)	0.10 (0.01)	0.10 (0.01)	0.10 (0.03)	0.10 (0.01)	0.30 (0.02)	0.70 (0.02)	0.10 (0.01)	0.10 (0.02)
C22:4($n-6$)	3.2 (0.1)	3.0 (0.1)	3.0 (0.1)	3.2 (0.1)	4.0 (0.1)	2.80 (0.02) [d]	1.8 (0.1) [f]	1.90 (0.03)
C22:5($n-6$)	0.70 (0.03)	0.60 (0.04)	0.90 (0.05)	0.80 (0.04)	7.7 (0.1) [f]	5.6 (0.1) [a]	0.20 (0.02) [f]	0.20 (0.01)
C22:5($n-3$)	–	–	0.10 (0.01)	0.10 (0.01)	–	–	0.50 (0.10)	0.50 (0.03)
C24:0	0.70 (0.04)	0.80 (0.07)	0.90 (0.04)	1.0 (0.0)	0.60 (0.03)	0.80 (0..01)	0.60 (0.03)	0.9 (0. 1)
C22:6($n-3$)	14.8 (0.2)	12.8 (0.3) [b]	13.7 (0.1)	14.0 (0.3)	8.4 (0.1) [f]	7.3 (0.2) [c]	16.5 (0.1) [f]	15.0 (0.1)

[a] $P < 0.001$ compared to the control group (Student's t test).
[b] $0.001 < P < 0.01$ compared to the control group (Student's t test).
[c] $0.01 < P < 0.02$ compared to the control group (Student's t test).
[d] $0.02 < P < 0.05$ compared to the control group (Student's t test).
[e] $0.001 < P < 0.01$ as compared to the standard diet.
[f] $P < 0.001$ as compared to the standard diet.

TABLE VI

Indices of lipid differences in synaptic membranes from rats fed different diets and intoxicated or not with ethanol

See legend to Table V. Values calculated from Table V. (DBI/S) = (% of each fatty acid × number of double bonds/fatty acids/% of saturated fatty acid); DBI, double bond index; S, saturated fatty acids; MUFA, monounsaturated fatty acids; and PUFA, polyunsaturated fatty acids

Diets:	Standard		Soya		Sunflower		Sunflower + CLO	
Treatment:	control (7)	ethanol (7)	control (5)	ethanol (5)	control (4)	ethanol (4)	control (7)	ethanol (7)
Σ S	44.8 (0.3)	46.0 (0.1) [b]	45.5 (0.3)	44.5 (0.1)	43.8 (0.5)	46.7 (0.1) [a]	43.9 (0.2)	44.1 (0.6)
Σ MUFA	22.8 (0.5)	23.3 (0.6)	22.3 (0.3)	22.8 (0.4)	21.3 (0.3)	23.0 (0.1) [b]	25.1 (0.6) [b]	26.1 (0.8)
Σ(n − 6)	15.1 (0.3)	14.2 (0.2) [d]	15.1 (0.3)	15.2 (0.5)	22.9 (0.2) [f]	18.4 (0.3) [a]	12.0 (0.2) [f]	12.2 (1.4)
Σ(n − 3)	14.8 (0.3)	12.8 (0.3) [b]	13.7 (0.1)	14.0 (0.3)	8.4 (0.1) [f]	7.3 (0.2) [c]	17.0 (0.1) [e]	15.5 (0.9)
Σ PUFA	29.9 (0.4)	27.0 (0.8) [c]	28.8 (0.6)	29.2 (0.5)	31.3 (0.7)	25.7 (0.8) [b]	28.5 (0.6)	27.7 (1.3)
(n − 6)/(n − 3)	1.00 (0.02)	1.10 (0.03)	1.10 (0.04)	1.06 (0.03)	2.7 (0.1) [f]	2.5 (0.1)	0.70 (0.01) [f]	0.78 (0.03)
DBI/S	3.8 (0.1)	3.3 (0.1) [a]	3.7 (0.1)	3.8 (0.1)	3.8 (0.1)	3.0 (0.1) [a]	3.9 (0.1)	3.7 (0.2)
C20:3(n − 6) / C18:2(n − 6)	0.56 (0.02)	0.34 (0.05) [b]	0.22 (0.04)	0.24 (0.02)	0.49 (0.04)	0.34 (0.01) [c]	0.73 (0.07)	0.58 (0.06) [d]
C20:4(n − 6) / C20:3(n − 6)	20.0 (3.3)	22.0 (2.2)	49.0 (1.0)	46.2 (2.0)	33.8 (0.5)	28.0 (0.4) [d]	14.3 (0.3)	13.7 (0.5)
C20:4(n − 6) / C18:2(n − 6)	11.0 (1.0)	7.5 [b] (0.6)	11.0 (1.1)	11.0 (1.2)	16.8 (1.1)	9.3 (0.3) [b]	10.5 (1.1)	7.4 (0.5) [d]

altered. The animals fed the diet containing sunflower + CLO exhibited a decrease in the proportion of the major PUFA of the $(n-6)$ series [C20:4$(n-6)$, C22:4$(n-6)$, C22:5$(n-6)$] and an increase in those of $(n-3)$ series [C22:5$(n-3)$, C22:6$(n-3)$] resulting in a decreased $(n-6)/(n-3)$ ratio (0.7 vs. 1.0 and 1.1 in SPM of rat fed standard diet and soya oil diet, respectively). A significant increase was seen in C16:1$(n-7)$ resulting in increased MUFA content. The total amount of the polyunsaturated and saturated fatty acids and the DBI/S ratio were not altered (Table V and VI). No significant effects of dietary fat supply were seen on the amount of synaptic membrane phospholipids and cholesterol or on the cholesterol/phospholipids molar ratio in animals fed the diets containing soya, sunflower or sunflower + CLO as compared to those fed the standard diet (Table VII).

Effect of dietary fat together with chronic ethanol consumption on the lipid composition of synaptic membranes

With rats fed the standard diet, the percentage of C14:0, C16:0, C18:1$(n-9)$ and C18:2$(n-6)$ were increased in response to ethanol consumption while

TABLE VII

Concentrations of cholesterol and phospholipids in synaptic membranes from rats fed different diets and intoxicated or not with ethanol

See legend to Table V

Diets:	Standard		Soya		Sunflower		Sunflower + Cod liver oil	
Treatment:	control (7)	ethanol (7)	control (5)	ethanol (5)	control (5)	ethanol (5)	control (5)	ethanol (5)
Cholesterol (nmol/mg protein)	361 (12)	445 (8) *	294 (5)	373 (4) *	309 (9)	317 (25)	325 (15)	305 (20)
Phospholipids (nmol/mg protein)	630 (31)	634 (39)	551 (7)	598 (3)	523 (14)	528 (29)	519 (31)	478 (25)
Cholesterol/Phospholipids (molar ratio)	0.57 (0.04)	0.71 (0.03) **	0.57 (0.01)	0.65 (0.02) **	0.60 (0.05)	0.61 (0.03)	0.62 (0.03)	0.64 (0.02)

* $0.001 < P < 0.01$

** $P < 0.001$, compared to control group.

C20:4($n-6$) and C22:($6n-3$) were decreased. As a result, the total amount of saturated fatty acid content was increased and that of PUFA decreased, with a decrease of the DBI/S ratio. The $(n-6)/(n-3)$ molar ratio was not altered. The cholesterol content and the cholesterol/phospholipids molar ratio were increased. The effects of ethanol on synaptic membranes of rats fed the sunflower oil diet were more marked. The major PUFA were altered: C18:2($n-6$) was increased, whereas C20:4($n-6$), C22:4($n-6$), C22:5($n-6$) and C22:6($n-3$) were decreased. C14:0 and C18:1($n-9$) were altered with an increase in MUFA and saturated fatty acid amounts. The $(n-6)/(n-3)$ ratio was not affected while the DBI/S ratio was reduced. No difference was seen in the cholesterol content or in the cholesterol/phospholipid ratio. There were fewer changes in lipid composition of synaptic membranes of rats fed the sunflower oil supplemented with CLO diet: C18:1($n-9$) and C18:2($n-6$) were increased. A slight but not significant decrease in C22:6($n-3$) was seen with an increase in $(n-6)/(n-3)$ ratio. No variation was observed in saturated fatty acids, MUFA, PUFA or in the cholesterol/phospholipids ratio. Minimal changes were seen in the fatty acid composition of the synaptic membrane between treated and control rats fed with the soya oil diet except for C18:1($n-9$) which was increased significantly, nevertheless cholesterol content and cholesterol/phospholipids were increased (Table VII).

Effect of dietary fat on the baseline degree of fluorescence polarization of DPH probes in the synaptic membranes

No difference in the intrinsic degree of fluorescence polarization (P) could be found with the DPH probe in SPM of rats fed either the soya or the sunflower + CLO diet, while this parameter was significantly lower in SPM of rats fed sunflower oil diet as compared to rats fed standard diet. We have previously noted [26] the increase of 'fluidity' of the SPM from rats fed the sunflower oil diet. Studies with both TMA-DPH and PROP-DPH showed no difference in the intrinsic P of the SPM from rats fed an experimental diet as compared to those fed the standard diet. The P of the different probes in the SPM were clearly dissimilar: P(PROP-DPH) $>P$(TMA-DPH) $>P$(DPH). These results are consistent with those of other authors and indicate that the membrane surface is less 'fluid' (more organized) than the lipid core. The P of the PROP-DPH probe was significantly higher than that of TMA-DPH. The two compounds preferentially probe different microdomains of the membrane surface and perhaps different leaflets [13] (Table VIII).

Effects of dietary fat together with chronic ethanol treatment on the baseline degree of fluorescence polarization of the synaptic membranes

Following the chronic ethanol treatment, SPM from rats fed the standard, sunflower or sunflower + CLO diets exhibited a decrease of the 'fluidity' of the lipid core, while no difference was shown with the soya oil diet group. Studies with the TMA-DPH probe showed that the polar region of the SPM from the sunflower treated rats was less fluid compared to control, while no difference was shown in SPM of rats fed the three other diets. The balance between ($n-6$) and ($n-3$) PUFA levels could play a role at the membrane polar level, giving a low 'fluidity' in this region with the sunflower oil diet. Using the PROP-DPH probe, no difference of P was found between the ethanol treated rats and controls whatever the diet used (Table VIII).

The P results at the apolar membrane level are slightly at variance with those obtained previously with the same ethanol treatment [27] which did not give any statistically significant increase in P(DPH) with the ethanol-treated rats as compared to controls. Never-

TABLE VIII

Degrees of fluorescence polarization of DPH probes incorporated into synaptic membranes from rats fed different diets and intoxicated or not with ethanol

Values are means ± S.E. of results obtained from (n) animals. See legend to Table V. Two comparisons are shown: (1) Treated rats to control ones for the same diet (letters used a–d as for Table V); (2) Rats fed experimental diet to rats fed standard diet (letter used, f, as for Table V). [g] $0.001 < P < 0.01$ as compared to the P(TMA-DPH) for the same diet. [h] $P < 0.001$ as compared to the P(TMA-DPH) for the same diet.

Diets:	Standard		Soya		Sunflower		Sunflower + CLO	
Treatment:	control (5)	ethanol (5)	control (4)	ethanol (4)	control (5)	ethanol (5)	control (7)	ethanol (7)
P(DPH)	0.336 (0.002)	0.347 (0.002) [a]	0.336 (0.002)	0.337 (0.004)	0.327 (0.001) [f]	0.339 (0.004) [c]	0.330 (0.007)	0.343 (0.003) [c]
P(TMA-DPH)	0.359 (0.006)	0.351 (0.002)	0.350 (0.004)	0.351 (0.005)	0.357 (0.003)	0.364 (0.003) [d]	0.352 (0.007)	0.351 (0.011)
P(PROP-DPH)	0.368 (0.004)	0.374 (0.004) [g]	0.372 (0.002) [g]	0.373 (0.003) [g]	0.372 (0.005) [h]	0.377 (0.001) [h]	0.369 (0.005) [g]	0.376 (0.006) [g]

theless, as the lab chow used in these previous studies was slightly different from the standard diet presently used in this study, this discrepancy emphasizes the importance of the dietary fatty acid supply in the membrane responses to ethanol.

Discussion

The present work confirms that the brain, especially at the nerve endings (synaptosomes), is more resistant than other organs to dietary lipid-induced changes in fatty acid composition. Nevertheless, this study shows clear differences in response to ethanol intoxication of rats receiving different dietary PUFA with respect to the content of cholesterol and the composition of the fatty acids in their SPM.

A very efficient homeostasis is operative in biological membranes and may act as a buffer to protect membranes from the effects of changes in the nature of the dietary lipid intake [28] and result in an apparent homeoviscosity. The potential effects of dietary lipids on various physiological processes should certainly be protected [29,30]. Nevertheless, a real ($n-3$) deficiency as induced by the sunflower oil diet leads to the most clear-cut changes in the SPM fatty acid profile with a low amount of (C22:6($n-3$)) compensated for by an increase in (C22:5($n-6$)), according to a previous study [1]. When the sunflower oil diet is supplemented with cod liver oil, the situation is quite different, with a high level of ($n-3$) PUFA, and low one in the ($n-6$) PUFA amount . The decrease of C20:4($n-6$)/C20:3($n-6$) ratio indicates an inhibition of the activity of Δ^5 desaturase [31]. These results go with those found in a previous work for all brain [32].

The nature of the relationship between the level of unsaturated fatty acids or the DBI and the so called 'fluidity' of the membrane still remains unclear [29]. It is now assumed that the relationship is far from a simple one [33]; this study clearly demonstrated it. Although the fatty acids amount was not significantly different in the SPM of rats fed the different diets as well as another potential 'fluidity' determinant: Ch/PL [34], the SPM from the animals fed the sunflower oil diet displayed a more fluid membrane lipid core, in agreement with our previous work [26]. The difference of conformation between the ($n-3$) and ($n-6$) PUFA in the membrane certainly plays a role: a high level in ($n-6$) PUFA giving a less packed (more fluid) lipid apolar core [35–37]. Except with the sunflower oil diet, the fine tuning of the membrane fatty acid profile is accompanied by an apparent maintenance of the overall 'fluidity'. These results stress the importance of keeping the membrane physical state within the narrow limits necessary for proper functioning [38–40].

Despite these limited diet-related alterations in the synaptic membranes, clear-cut differences were noted in response to ethanol intoxication. Changes in membrane lipid composition after ethanol intoxication have been extensively studied [3,41] in relation to the membrane microorganization and the mechanisms underlying tolerance and dependence. At the brain membrane level, there is some evidence of consistent alterations in the composition of PUFA after ethanol exposure in rats and have been attributed to an enhanced lipid peroxidation [42], to a decrease in the desaturase processes [43] or to the stimulation of endogeneous phospholipase A_2 [44]. Different patterns of changes in fatty acids and in cholesterol content were, however, found depending on the animal species considered, on the membrane types, on the classes of phospholipids studied and also on the type and duration of ethanol administration and on the techniques used [45]. This study demonstrated that the concurrently ingested diet could also play a non-negligible role. Under our experimental conditions, ethanol intoxication of the rats fed the standard diet induced most of the fatty acid alterations already described. The proportion of saturated fatty acids was increased while that of PUFA was decreased. The fact that the C20:3($n-6$)/C18:2($n-6$) ratio is decreased could indicate that $\Delta 6$ desaturase enzyme is inhibited.

The same but more marked fatty acid alterations after ethanol treatment were found in the rats fed the sunflower oil diet than those fed a standard diet. It has already been shown [26] that rats fed a sunflower-enriched diet are very sensitive to the effects of ethanol both at the membrane level as well as from a functional point of view [28]. The $\Delta 6$ and $\Delta 5$ desaturases could be inhibited as shown by a decrease in C20:3($n-6$)/C18:2($n-6$) and C20:4($n-6$)/C20:3($n-6$) ratios. Contrary to the standard diet results, the cholesterol content of the membranes and their Ch/PL ratio were not affected by the ethanol intoxication. When the sunflower oil diet was supplemented with long chain ($n-3$) PUFA with cod liver oil, the ethanol-induced perturbation in SPM fatty acids was partly prevented, C18:1($n-9$) was increased as well as C18:2($n-6$). The C20:3($n-6$)/C18:2($n-6$) ratio is markedly decreased. As with sunflower oil alone, neither the cholesterol concentration nor the Ch/PL ratio in the SPM were affected by ethanol treatment.

Though the soya oil diet gives a same fatty acid profile in SPM that obtained with the standard diet, ethanol exposure does not greatly affect it. In fact, the only significant change after ethanol was found with C18:1($n-9$). Indeed, an increase in C18:1($n-9$) has been consistently noted after ethanol intoxication in different organs, in man [46] as well as in animals [47]. In the present study, it was found in the SPM of rat whatever the associated diet. In fact it was well documented that $\Delta 9$ desaturase responds very rapidly to

dietary changes and that starving and refeeding induce its activity [32] as well as the the type of dietary carbohydrate [48]. Thus, feeding a diet rich but well balanced in $(n-3)$ and $(n-6)$ PUFA, as is the soya oil diet, can alleviate some of the deleterious effects of chronic ethanol intoxication on synaptic membrane PUFA. The apparent lipid insensibility to ethanol is however not total since cholesterol content and the Ch/PL ratio increase. PUFA and cholesterol have closely related metabolisms in the membrane [34]. Furthermore, response of the synaptic membrane 'fluidity' is subject to complex regulation and is not easily predictable from the variation in fatty acid profiles or in cholesterol concentrations [28].

Despite this exquisite regulation [29], we noted in this study that changes in P(DPH) (index of the 'fluidity' of the apolar part of the membrane) are even more marked than previously found with the same ethanol intoxication model. A rigidification or decreased 'fluidity' which has been described as a clue of the state of dependence to ethanol [8] was found with all the diets except the soya oil diet. In addition, P(DPH) was found to correlate negatively quite well with the overall PUFA level in the membrane ($r = -0.60$, $P < 0.01$ for the alcohol treated animals, $r = -0.58$, $P < 0.01$ for the control animals and $r = -0.65$, $P < 0.001$ for all data), although no correlation was found either with the amount of saturated fatty acids or with the DBI. This finding is in agreement with recent papers suggesting that PUFA have structurally specific roles depending on their peculiar double bond distribution [49] which gives them special conformations and thus greatly affect membrane properties [37].

Conclusion

Changes in dietary PUFA lead to more or less marked changes in the synaptic fatty acid composition which do not mirror the great differences in supply of these PUFA in the diet. Little impact was found on the 'fluidity' parameters of the brain synaptic membranes. The control mechanism of the 'fluidity' is definitely very strong and multifactorial.

Some studies have emphasized the positive action of fish oil in combating some of the adverse effects of ethanol intoxication, mainly on circulating lipids [50]. In the present study, even a low level of long chain $(n-3)$ PUFA has been found to be rather harmful at the membrane level. Further studies are needed to assess clearly the positive or negative interactions between fish oil supplementation and ethanol pathophysiological effects. A structurally specific role of PUFA is demonstrated by the negative effects of the C18:3$(n-3)$ deficient diet. The results obtained with a diet rich and well balanced in $(n-3)+(n-6)$ PUFA (soya oil diet) argue in that case in favour of some kind of protective effect against ethanol. Such research, already initiated [51], is important because of the possibility of antagonizing some of the toxic effects of alcohol on the cell membrane and especially the development of the physical membrane alterations associated with tolerance to and dependence on alcohol.

Acknowledgements

This work was supported by INSERM (U26), IREB (grant No. 88-09) and INRA.

References

1 Bourre, J.M., Pascal, G., Durand, G., Masson, M., Dumont, O. and Piciotti, M. (1984) J. Neurochem. 43, 342–348.
2 Brivio-Hangland, R.P., Louis, SL., Musen, K., Waldeck, N., William, MA. (1976) Biochim. Biophys. Acta 433, 150–163.
3 Leonard, B.E. (1986) Alcohol Alcohol. 21, 325–338.
4 Beaugé, F., Stibler, H. and Kalant, H. (1983) Pharmacol. Biochem. Behav. 18, Suppl. 1, 519–524.
5 Alling, C., Liljequist, S. and Engel, J. (1982) Med. Biol. 60, 149–154.
6 Harris, R.A., Baster, D.M., Mitchell, M.A. and Hitzemann, R.J. (1984) Molecular. Pharmacol. 25, 401–409.
7 Chin, J.H., Parsons, L.M and Goldstein, D.B. (1978) Biochim. Biophys. Acta 513, 358–363.
8 Rubin, E. and Rottenberg, H. (1983) Pharmacol. Biochem. Behav. 18, Suppl. 1, 7–13.
9 Polokoff, M.A., Simon, T.J., Harris, R.A., Simon, F.R. and Iwahashi, M. (1985) Biochemistry 24, 3114–3120.
10 Sun, G.Y. and Sun, A.Y. (1979) Res. Commun. Chem. Pathol. Pharmacol. 24, 405–408.
11 Lewtz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528.
12 Prendergast, F.G., Hangland, R.P. and Callahan, P.J. (1981) Biochemistry 20, 7333–7338.
13 Schroeder, F., Morrison, W.J., Gorka, C. and Wood, W.G. (1988) Biochim. Biophys. Acta 946, 85–94.
14 Guesnet, P., Pascal, G. and Durand, G. (1986) Reprod. Nutr. Develop. 25, 969–985.
15 Bourre, J.M., François, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G. and Durand, G. (1989) J. Nutr. 119, 1880–1892.
16 Durand, G., Guesnet, P, Desnoyers, F. and Pascal, G. (1986) Prog. Lipid Res. 25, 395–400.
17 Buege, J.A. and Aust, S.D. (1978) Methods Enzymol. 55, 302–310.
18 Leblanc, A.E., Kalant, H., Gibbins R.J. and Berman, N.D. (1969) J. Pharmacol. Exp. Ther. 168, 244–250.
19 Cotman, C.W. and Matthew, D.A. (1971) Biochim. Biophys. Acta 249, 380–394.
20 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
21 Rose, H.G. and Oklander, M. (1965) J. Lipid Res. 6, 428–431.
22 Siedel, J., Hägele, E.O., Ziegenhorn, J. and Wahlefeld, A.W. (1983) Clin. Chem. 29, 1075–1080.
23 Chen, P.S., Toribaca, T.Y. and Warner. H. (1956) Anal. Chem. 28, 1756–1758.
24 Morrisson, W.R. and Smith, L.M. (1964) J. Lipid Res. 5, 600–608.
25 Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657.
26 Beaugé, F., Zérouga, M., Niel, E., Durand, G. and Bourre, J.M. (1988) in Biomedical and social aspects of Alcohol and Alcoholism (Kuriyama, K., Takeda, A. and Ishii, H., eds.) pp. 291–294, Elsevier, Amsterdam.

27 Beaugé, F., Fleuret-Balter, C., Nordmann, J. and Nordmann, R. (1984) Alcohol.: Clin. Exp. Res. 8, 167–171.
28 Gibson, R.A., McMurchie, E.J., Charnock, J.S. and Kneebone, G.M. (1984) Lipids, 19, 942–951.
29 Murphy, M.G. (1990) J. Nutr. Biochem. 1, 68–79.
30 Casals, C., Henera, L., Gasset, M., Garcia Barreno, P. and Municio. A.M. (1989) Cell. Biochem. Funct. 7, 193–199.
31 Nassar, B.A., Huang, Y.S., Manku, M.S., Das, U.N., Morse, N. and Horrobin, D.F. (1986) Lipids 21, 652–657.
32 Bourre, J.M., Bonneil, M., Dumont, O., Piciotti, M., Calaf, R., Portugal, H., Nalbone, G. and Lafont, H. (1990) Biochim. Biophys. Acta 1043, 149–152.
33 Stubbs, C.D., (1989) in Biomembranes and Nutrition (Léger, C.L. and Béréziat, G., eds.), Vol. 195, pp. 125–134.
34 Van Blitterswijk, W.J., Wieb Van der Meer, B. and Hilkmann, H. (1987) Biochemistry 26, 1746–1756.
35 Ehringer, H., Belcher, D., Wassall, S.R. and Stillwell, W. (1990) Chem. Phys. Lipids 54, 79–88.
36 Belcher, D., Stillwell, W., Langsford, C.A. and Wassall, S.R. (1988) Biochem. Biophys. Res. Commun. 150, 1063–1068.
37 Rajamoorthi, K., Trouard, T.P., Salmon, A. and Brown, M.F. (1990) Biophys. J. 57, 273.
38 Engelhard, V.H., Esko, J.D., Storm, D.R. and Laser, M. (1976) Proc. Natl. Acad. Sci. USA 73, 4482–4486.
39 Ginsberg, B.H., Jabour, J. and Spector, A.A. (1982) Biochim. Biophys. Acta 690, 157–164.
40 Balcar, V.J., Robert, J. and Mandel, P. (1980) J. Neurochem. 34, 1678–1681.
41 Sun, G.Y. and Sun, A.Y. (1985) Alcohol. Clin. Exp. Res. 9, 164–180..
42 Reinke, L.A. (1990) Free Rad. Comms. 9, 205–211.
43 Wang, D.L. and Reitz, R.C. (1983) Alcohol. Clin. Exp. Res. 7, 220–226.
44 Salem, J. and Karanian, J.W. (1988) Advances in Alcohol and Substance Abuse, 7, 183–197.
45 Aloia, R.C., Paxton, J., Daviau, J.S., Van Gelb, O., Mlekusch, W., Truppe, W., Meyer, J.A. and Brauer, F.S. (1985) Life Sci. 36, 1003–1017.
46 Alling, C., Gustavsson, L., Kristensson A. and Wallerstedt, S. (1984) Scand. J. Clin. Lab. Invest. 44, 283–289.
47 Cunningham, C., Filus, S., Bottenus, R. and Spach, P. (1982) Biochim. Biophys. Acta 712, 225–233.
48 Peluffo, R.O., Nervi, M.S., Gonzalez, M.S. and Brenner, R.R. (1984) Lipids, 19, 154–157.
49 Léger, C.L., Davelosse, D., Christon, R. and Viret, J. (1990) Biochemistry 29, 7269–7275.
50 Nestel, P.J., Connor, W.E. Reardon, M.F., Connor, S., Wong, S. and Boston, R. (1984) J. Clin. Invest. 74, 82–89.
51 Glen, I., Skinner, F., Glen, E. and MacDonell, L. (1987) Alcohol.: Clin. Exp. Res. 11, 37–41.