Diet-induced structural and functional modifications in the pig liver endoplasmic reticulum membrane: effect of polyunsaturated fatty acid deficiency

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Weaned 39-day-old female piglets were fed diets containing either corn-soybean oil (control) or hydrogenated coconut oil (essential fatty acid (EFA) deficient) for 12 weeks. EFA deficiency produced increased cholesterol (Chol) and decreased phospholipid (PL) contents relative to protein, and thereafter an enhanced Chol/PL molar ratio in the liver endoplasmic reticulum (E.R.) membrane. The primary changes included modified PL distribution, with significant decrease in phosphatidylcholine (PC) and increase in phosphatidylinositol (PI) and phosphatidylserine (PS) levels. Furthermore, fatty acid profiles of the four main PL classes were altered by EFA deficiency. In particular, linoleic, arachidonic, and docosapentaenoic acid levels were largely reduced, whereas palmitoleic and oleic acid levels were increased. The biosynthesis of 5, 8, 11-eicosatrienoic acid (from oleic acid) was strongly stimulated in EFA-deficient pig liver E.R. membranes. The lower polyunsaturated fatty acid level of these latter induced a decreased peroxidability as measured by thiobarbituric assay. Moreover, the modified lipid composition due to EFA deficiency was followed by a decrease in membrane fluidity and an alteration in the activity of several membrane proteins. Liver E.R. membrane from EFA-deficient piglets exhibited a decreased Ca^{++} uptake, although passive Ca^{++} efflux was unaffected. NADH-Cyt.b_5 and NADH-Cyt.b_5 reductase activity were enhanced, whereas NADPH-Cyt. P450 electron transferring system and some of the liver detoxifying enzyme activities were significantly depressed.

Keywords: polyunsaturated (essential) fatty acids; membrane; endoplasmic reticulum enzyme activities; calcium uptake; lipid composition; membrane fluidity; pig

Introduction

Dietary polyunsaturated fatty acids (PUFA) are known to influence cell membrane lipid composition, which, in turn, determines membrane physicochemical properties and then membrane bound protein activities.[1,2] Particularly, the liver microsomal mixed-function-oxidase (MFO) system of rats has been shown to be stimulated by dietary PUFA.[3] This enzyme system consists of protein molecules more or less embedded in the membrane, forming two electron-transport chains, one of which is NADPH dependent (NADPH-cyt.c reductase, cytochrome P450) and essentially involved in xenobiotic metabolism, while the other is NADH dependent (NADH-cyt.b_5 reductase, cytochrome b_5) and is involved in fatty acid desaturation.[4,5] We recently have shown that a dietary essential fatty acid (EFA) deficiency caused several alterations in the endoplasmic reticulum membrane properties of rat liver cells.[6] In these experiments, EFA deficiency was induced by a hydrogenated coconut oil-based diet while control animals received a corn oil-based diet.

Moreover, the pig could be a good model animal

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for research on human diseases,[7] especially for studies on liver microsomal monooxigenase activity.[8] However, in this species, little is known about the influence of PUFA on the metabolic reactions in the liver endoplasmic reticulum (E.R.) membrane. More generally, the mechanisms involved in all of these processes are still not understood. Therefore, the present study was undertaken in the pig to determine the changes in the hepatic E.R. membrane properties and functions due to a total PUFA deficiency.

Materials and methods

Animals, diets, and membrane preparation

Eight 39-day-old female Large-White piglets weaned at 21 days of age were divided into two homogeneous groups on a litter, age, and weight basis. The pigs were housed individually and received a balanced semi-synthetic diet consisting of casein (20%), cassava meal (25%), potato starch (30%), molasses (3.65%), cellulose (10%), dicalcium phosphate (1.7%), calcium carbonate (1.7%), sodium chloride (0.45%), trace mineral and vitamin mixture (0.5%), and either 7% corn and soybean oil mixture (1:1, vol/vol) (Control), or 7% hydrogenated coconut oil (EFA-deficient) for 12 wk. The mineral and vitamin mixture provided (in mg/kg diet): $ZnSO_4 \cdot 7H_2O$, 44; $MnSO_4 \cdot H_2O$, 6; $CuSO_4 \cdot 5H_2O$, 3; KI, 0.1; $CoSO_4$; KI, 0.1; $CoSO_4$, 0.1; Na_2Se, 0.1; vitamin A (500.000 I.U./g), 1.3; vitamin D_3 (100.000 I.U./g), 1.3; vitamin E (500 I.U./g), 2.5; vitamin K_3, 0.3; thiamin, 0.1; riboflavin, 0.5; pyridoxine hydrochloride, 0.1; nicotinic acid, 1.9; folic acid, 0.1; vitamin B_{12}, 5×10^{-3}; biotin, 25×10^{-3}; calcium pantothenate, 1.3; and choline, 63. The control and EFA-deficient diets only differed by the fatty acid composition of their lipid contents *(Table 1)*. The control diet contained 4028 mg of linoleic acid and 301 mg of linolenic acid [18:3 (n-3)] per 100 g, whereas the EFA-deficient diet was a highly saturated lipid diet containing only 48 mg of 18:2 (n-6) and 14 mg of 18:3 (n-3) per 100 g. Each pig received the same amount of food (on a body wt basis), while water was supplied ad libitum.

Table 1 Fatty acid composition of dietary lipids[a]

Fatty acid[b]	Control	EFA-deficient
8:0	—	1.2
10:0	—	4.2
12:0	—	46.5
14:0	2.1	20.2
16:0	11.2	11.5
18:0	2.9	14.7
16:1 (n-7)	0.1	—
18:1 (n-9)	20.3	0.8
18:2 (n-6)	59.0	0.7
18:3 (n-3)	4.4	0.2
Saturated	16.2	98.3
Monounsaturated	20.4	0.8
n-6 polyunsaturated	59.0	0.7
n-3 polyunsaturated	4.4	0.2
n-6/n-3	13.4	3.5

[a]Lipid supply of the diet was 7.2% by weight.
[b]Fatty acids (wt % of total) were designated by the number of carbon atoms followed by the number of double bonds. The position of the first double bond relative to the methyl (n) end of the molecule was also indicated. Fatty acids contributing less than 0.1% were omitted (—).

At the end of the experimental period, the animals were fasted for 18 hr and killed by electrical stunning and exsanguination. Blood was collected and the liver rapidly removed. A 10% liver homogenate was then prepared in ice-cold 3 mmol/L EDTA, 154 mmol/L KCl at pH 7.4, and the microsomes were isolated by the technique of Lowrey et al.[9] The pellet was resuspended in ice-cold buffer (50 mmol/L Tris, 50 mmol/L maleate, 100 mmol/L KCl, pH 7.4) and the membrane vesicles were frozen and stored in liquid nitrogen until analysis. Protein was estimated according to Lowry et al.,[10] using bovine serum albumin as a standard.

Membrane lipid composition

Lipid composition of microsomal membrane vesicles was determined as previously described.[11] After extraction of the total lipids using chloroform/methanol (2:1, vol/vol) solvent containing 0.01% (wt/vol) butylated hydroxytoluene, neutral lipids, glycolipids, and phospholipids were separated by a Sep-Pak method. Cholesterol was assessed from the neutral lipid fraction by an enzymatic method (Boehringer Mannheim, Meylan, France). Phospholipid classes were separated by high-performance liquid chromatography (Beckman Instruments, Gagny, France); their purity was checked using thin layer chromatography and quantitative estimation was done by determination of inorganic phosphorus. Total phospholipids were then transmethylated and the fatty acid methyl esters obtained were analyzed using a Packard model 427 gas chromatograph (Packard, Rungis, France) equipped with a flame ionization detector and a CP WAX 52 CB bonded fused silica capillary column (50 m × 0.2 mm I.D.). Peaks were identified by comparison of equivalent chain lengths with those of authentic fatty acid methyl esters. Automated expression of data was performed by a microcomputer coupled with a Delsi (ENICA 10) integrator (Delsi Instruments, Suresnes, France).

Electron spin resonance (ESR) technique

Spin-labeled microsomes were prepared using stearic acids with a doxyl group attached to either carbon atom 5 (5-doxylstearic acid), 12 (12-doxylstearic acid), or 16 (16-doxylstearic acid), according to Utsumi et al.[12] The spin-label:microsomal lipid molar ratio was about 1:200 and the protein concentration was 200 mg/mL. The spectra were recorded with an ER 200 D Brucker-X band spectrometer (Brucker Spectro Spin S.A., Vissembourg, France). The temperature was monitored at 37° C with a thermocouple and maintained by using a chilled or heated N_2 gas flow system. All samples were equilibrated for 10 min in the cavity prior to spectra recording. The order parameter S (for 5-doxylstearic acid) and the rotational correlation time τ_c (for 12- and 16-doxylstearic acids) were calculated according to Hauser et al.[13]

Nonenzymatic lipid peroxidation

Lipid peroxidation level was measured in reaction mixture containing 0.1 mg of microsomal proteins, 100 μmol/L $FeCL_3$ and 100 μmol/L ascorbate[14] in a final volume of 1 mL, 50 mmol/L Tris, 50 mmol/L maleate, 100 mmol/L KCl, pH 7.4. After a 30 min incubation period at 37° C, 0.5 mL ice-cold trichloroacetic acid solution (25%) was added. The mixture was centrifuged and malondialdehyde (MDA) present in the supernatant was determined by the thiobarbituric acid (TBA) assay.[15]

Ca^{++} uptake and efflux

Ca^{++} uptake by the membrane vesicles was determined following the method of Moore et al.[16] The reaction medium contained: 5 mmol/L ATP, 20 μmol/L $CaCl_2$, 0.1 μCi ^{45}Ca/mL, 5 mmol/L $MgCl_2$, 5 mmol/L NaN_3, 5 mmol/L ammonium oxalate, 0.1 mg of microsomal proteins/mL in 30 mmol/L histidine, 30 mmol/L imidazole and 100 mmol/L KCl buffer pH 6.8. After incubation at 37° C, 0.5 mL samples were filtered on prewetted cellulose nitrate filters (pore size 0.45 μm) and washed with 10 mL ice-cold buffer. The radioactivity was monitored in a liquid scintillation counter.

Measurements of Ca^{++} premeability were performed in two stages.[17] First, microsomes were preloaded for 6 min at 37° C in the reported incubation system in which the ^{45}Ca activity was 0.5 μCi/mL and the protein concentration was 1 mg/mL and oxalate was omitted. Ca^{++} accumulated in membrane vesicles after 6 min was as follows: 25 μL were removed from the loading suspension, filtered, washed, and counted as described. Then the reactional medium was diluted 20-fold with buffer and several 0.5 mL samples were taken between 0.25 and 10 min. The presented values take into account the corresponding results obtained without ATP. Kinetics of Ca^{++} efflux were plotted according to Cauvin et al.[18] to allow determination of the rate constants.

Activities of mixed-function-oxidase system

Cytochrome b_5 and total cytochrome P450 levels were determined according to Omura and Sato[19] with respective molecular extinction coefficients of 171 cm^{-1} $mmol/L^{-1}$ and 91 cm^{-1} $mmol/L^{-1}$. NADPH-cytochrome c reductase and NADH cytochrome b_5 reductase activities were measured in the presence of cytochrome c and ferricyanide, respectively, according to Hernandez et al.[20] as modified by Strobel and Dignam[21] and Hrycay and Prough.[22] Microsomal N-demethylation of aminopyrine was measured by colorimetric determination of formaldehyde by Nash reactive[23] as described by Mazel.[24] Aniline hydroxylase activity was estimated by measuring the amount of para-aminophenol formed, by the method of Imaï et al.[25] Incubations were made in 0.33 mmol/L $MgCl_2$, 7.5 mmol/L NADP, 1.25 mmol/L glucose-6-phosphate dehydrogenase, 50 mmol/L Hepes buffer, pH 7.4 and 0.2 mmol/L aminopyrine or 0.4 mmol/L aniline.[26] Samples of 0.6 mL containing 1.0–1.2 mg of microsomal proteins were incubated at 37° C for 30 min. Benzopyrene hydroxylation was estimated according to Tulliez and Durand.[27]

Statistical analysis

Results are expressed as means ± SEM. Comparisons of mean values were made using Student's *t*-test ($P < 0.05$ considered significant).

Table 2 Feed intake and growth rate of piglets[a]

	Control	EFA-deficient
Initial body weight, kg	10.3 ± 0.2	9.4 ± 0.8
Final body weight, kg	62.1 ± 1.8	58.8 ± 2.2
Liver fresh weight :		
g	856.1 ± 30.3	890.5 ± 42.7
% body weight	1.4 ± 0.4	1.5 ± 0.5
Food intake, g/d	1103.3 ± 17.2	1061.3 ± 20.6
Growth rate, g/d	568.7 ± 17.1	538.7 ± 16.9

[a]Data are means ± SEM ($n = 4$). No significant difference was observed between dietary groups.

Table 3 Lipid composition of pig liver microsomal membrane[a]

	Control	EFA-deficient
Proteins (mg/g fresh liver)	26.8 ± 1.0	24.6 ± 0.8
Cholesterol (nmol/mg prot) :		
—free	259.2 ± 11.4	294.8 ± 10.0[b]
—esterified	23.6 ± 6.2	31.8 ± 9.2
Phospholipids :		
—total (nmol/mg prot)	508.0 ± 34.7	356.6 ± 30.8[c]
—classes (% of total):		
phosphatidylcholine (PC)	61.2 ± 0.9	52.6 ± 4.0[b]
phosphatidyl-ethanolamine (PE)	23.0 ± 0.4	25.1 ± 2.0
phosphatidylinositol (PI)	8.1 ± 0.1	10.4 ± 0.8[b]
phosphatidylserine (PS)	3.9 ± 0.4	6.2 ± 1.0[b]
sphingomyelin (SM)	1.3 ± 0.2	2.0 ± 0.2
diphosphatidyl-glycerol (DPG)	2.0 ± 0.5	2.8 ± 0.6
lysophosphatidyl-choline (LPC)	0.7 ± 0.1	1.4 ± 0.4
Cholesterol/phospho-lipids (mol/mol)	0.56 ± 0.04	0.93 ± 0.09[b]
SM/PC (mol/mol)	0.02 ± 0.00	0.04 ± 0.01[c]

[a]Values are means ± SEM for four pigs per dietary group.
[b]Different from control, $P < 0.05$.
[c]Different from control, $P < 0.01$.

Results

Pig growth

At the end of the experimental period, all animals appeared healthy. As shown in *Table 2,* food intake was similar for EFA-deficient and control pigs, and the dietary treatment had no significant effect on body weight or growth rate.

Cholesterol and phospholipid contents of liver microsomal membrane

The total cholesterol (nmol/mg protein) of liver microsomes was significantly higher in EFA-deficient pigs than in control pigs *(Table 3);* this increase was mainly free cholesterol, as esterified cholesterol remained unchanged.

Feeding the EFA-deficient diet also led to a decreased phospholipid (PL) level ($P < 0.01$) and altered phospholipid composition in the pig liver microsomal membrane. The proportion of phosphatidylcholine (PC) was significantly lower, and that of phosphatidylinositol (PI) and phosphatidylserine (PS) higher in EFA-deficient as compared with control pig membranes. Consequently, cholesterol/phospholipid (Chol/PL) and sphingomyelin (SM)/PC molar ratios were significantly increased by the dietary treatment.

Fatty acid composition of plasma and microsomal membrane phospholipids

The fatty acid composition of total phospholipids in plasma and liver microsomal membrane *(Table 4)* was greatly modified by EFA deficiency. Unsaturated fatty acids (UFA) were essentially changed, whereas the saturated fatty acid (SFA) levels remained unaffected. In both plasma and microsomes, the amounts of mono-UFA (MUFA) and 5, 8, 11-eicosatrienoic acid [ETA, 20:3 (n-9)] markedly increased ($P < 0.05$ or $P < 0.01$) in EFA-deficient pigs. By contrast, n-6 PUFA were significantly lowered, particularly linoleic, arachidonic, and docosatetraenoic [22:4 (n-6)] acids. The amounts of total n-3 PUFA and especially that of docosapentaenoic acid [22:5 (n-3)] were decreased ($P < 0.05$), while the eicosapentaenoic acid [EPA, 20:5 (n-3)] level was increased ($P < 0.01$), although not significantly in the liver microsomal total phospholipids. The 20:3 (n-9)/20:4 (n-6) ratio was significantly increased, expressing the EFA deficiency in the piglets.[28]

As SFA levels were also not changed in the PL main classes, only the main UFA of control and EFA-deficient pig liver microsomal membranes were shown in *Figure 1*. In all the PL classes the changes observed are generally the same as in microsomal total PL, particularly for MUFA and n-6 PUFA, although the rates of increase in 18:1 (n-9) and of decrease in 18:2 (n-6) were notably higher in PC than in the other PL classes, and that of decrease in 20:4 (n-6) was similar in PC and PI and higher than in PE and PS. The variations in n-3 PUFA levels differed between PL classes. There was a general decrease in 22:5 (n-3) level, only significant, however, in PC ($P < 0.01$) and phosphatidylethanolamine (PE) ($P < 0.05$). In these two major classes, a significant increase in the amount of EPA was observed, while the docosahexaenoic acid [DHA, 22:6 (n-3)] level was not modified in PC and PI, but enhanced ($P < 0.05$) in PE and PS. Total n-3 PUFA level significantly decreased in PC (3.0 ± 0.6 versus 4.0 ± 0.3) and did not change in the other PL classes of EFA-deficient pig liver microsomal membrane.

Spin-labeling

Values of physical parameters related to membrane fluidity are recorded in *Table 5*. Neither the order parameter S, at the C5 level, nor the rotational correlation time τ_c at the C16 level were significantly modified in EFA-deficient pig liver microsomes. However, with the 12-DS spin label, the values of τ_c were

Table 4 Fatty acid composition of pig phospholipids

Fatty acids[a]	Plasma		Liver microsomes	
	Control	EFA-deficient	Control	EFA-deficient
16:0	16.3 ± 0.6	18.8 ± 0.4[b]	12.8 ± 0.4	14.4 ± 0.6
18:0	26.2 ± 3.3	24.4 ± 0.7	30.0 ± 1.9	28.7 ± 0.4
20:0	0.4 ± 0.0	0.4 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
22:0	0.4 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.4 ± 0.2
24:0	0.7 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.5 ± 0.1
Σ SFA	**44.6 ± 3.3**	**45.3 ± 0.2**	**43.8 ± 1.6**	**44.8 ± 0.5**
16:1 (n-7)	0.4 ± 0.0	1.8 ± 0.2[c]	0.2 ± 0.1	1.8 ± 0.1[c]
18:1 (n-7)	1.3 ± 0.0	2.6 ± 0.1[c]	1.2 ± 0.1	2.6 ± 0.2[c]
16:1 (n-9)	0.4 ± 0.1	0.6 ± 0.0[b]	0.3 ± 0.0	0.7 ± 0.1[b]
18:1 (n-9)	12.7 ± 1.5	25.2 ± 0.4[c]	6.9 ± 0.2	17.5 ± 1.1[c]
20:1 (n-9)	0.2 ± 0.0	0.1 ± 0.0	—	0.1 ± 0.0
24:1 (n-9)	0.6 ± 0.0	0.5 ± 0.2	0.2 ± 0.0	0.2 ± 0.1
Σ MUFA	**15.6 ± 1.6**	**30.8 ± 0.7[c]**	**8.7 ± 0.3**	**22.9 ± 1.3[c]**
18:2 (n-6)	20.3 ± 2.4	5.5 ± 0.4[c]	20.2 ± 0.4	7.3 ± 0.8[c]
20:2 (n-6)	0.6 ± 0.1	—	0.5 ± 0.1	—
20:3 (n-6)	0.6 ± 0.1	0.5 ± 0.0	0.6 ± 0.1	0.5 ± 0.0
20:4 (n-6)	13.5 ± 1.0	4.8 ± 0.3[c]	21.0 ± 0.9	10.4 ± 0.6[c]
22:4 (n-6)	0.8 ± 0.1	0.3 ± 0.1[c]	0.7 ± 0.1	0.2 ± 0.1[c]
22:5 (n-6)	0.1 ± 0.1	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
Σ n-6 PUFA	**35.9 ± 1.9**	**11.4 ± 0.7[c]**	**43.0 ± 1.3**	**18.6 ± 1.3[c]**
18:3 (n-3)	0.2 ± 0.2	—	0.2 ± 0.1	0.1 ± 0.0
20:5 (n-3)	0.4 ± 0.0	0.8 ± 0.0[c]	0.1 ± 0.1	0.6 ± 0.3
22:5 (n-3)	2.0 ± 0.2	1.0 ± 0.0[b]	2.5 ± 0.2	1.5 ± 0.1[b]
22:6 (n-3)	0.9 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.1
Σ n-3 PUFA	**3.4 ± 0.2**	**2.8 ± 0.2[b]**	**4.1 ± 0.1**	**3.6 ± 0.3[b]**
Σ n-6 + n-3	39.3 ± 1.9	14.2 ± 0.9[c]	47.1 ± 1.3	22.2 ± 1.1[c]
n-6/n-3	10.5 ± 0.6	4.0 ± 0.2[c]	10.6 ± 0.2	5.4 ± 0.8[c]
20:3 (n-9)	**0.6 ± 0.2**	**9.7 ± 0.6[c]**	**0.4 ± 0.1**	**10.1 ± 0.5[c]**
20:3 (n-9)/20:4 (n-6)	0.04 ± 0.01	2.05 ± 0.22[c]	0.02 ± 0.01	0.98 ± 0.09[c]
DBI/SFA	3.1 ± 0.3	2.4 ± 0.0[b]	3.7 ± 0.3	2.9 ± 0.1

[a]Fatty acids are designated as in *Table 1*. Values are means ± SEM for four pigs. SFA, saturated fatty acids, DBI, double bond index = Σ (mol % each unsaturated fatty acid × number of double bonds of the same fatty acid); DBI/SFA, unsaturation index; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids. Superscript letters indicate significant differences from control : b, $P < 0.05$ or c, $P < 0.01$.

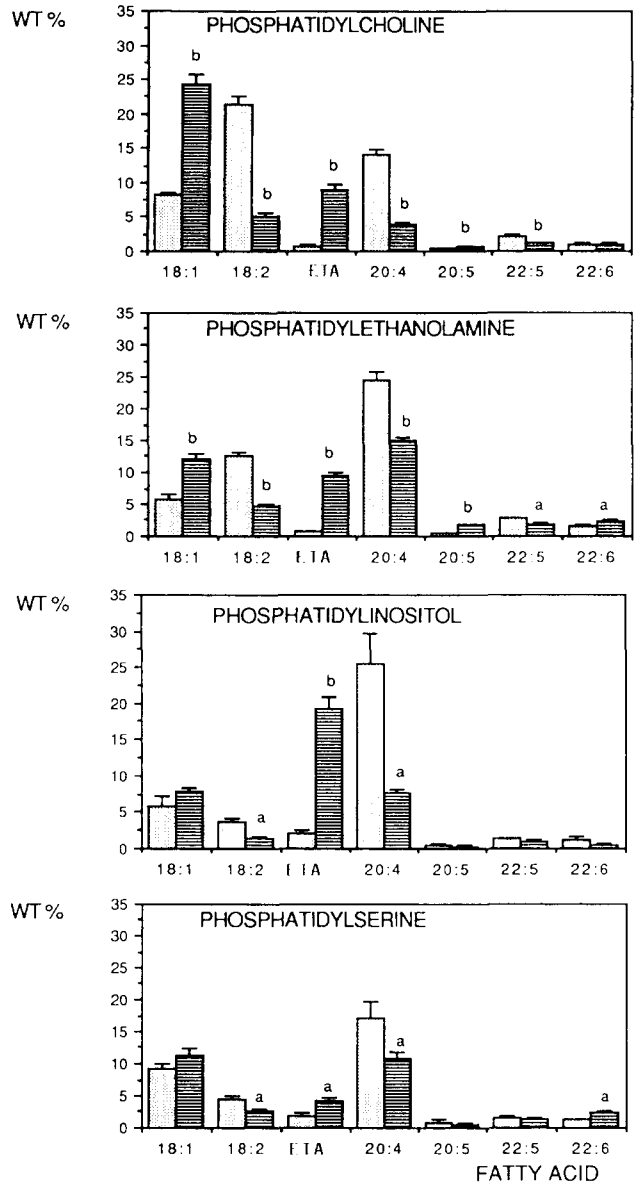

Figure 1 n-9 (18:1 and ETA), n-6 (18:2 and 20:4), and n-3 (20:5, 22:5, and 22:6) fatty acids (weight %) in the main phospholipids of the hepatic endoplasmic reticulum membrane of pigs fed either control (▨) or EFA-deficient (▤) diet. Values are mean ± SEM for four pigs. Superscript letters indicate significant difference from control: a, $P < 0.05$; b, $P < 0.01$.

Table 5 Order parameter S for 5-doxyl stearic acid and rotational correlation time τ_c for 12 and 16-doxyl stearic acids incorporated into microsomal membranes[a]

	Control	EFA-deficient
S (5-DS)	0.672 ± 0.005	0.682 ± 0.006
τ_c (12-DS) × 10^{10}s	30.67 ± 0.51	37.00 ± 0.47[b]
τ_c (16-DS) × 10^{10}s	14.89 ± 0.98	14.61 ± 0.14

[a]Values are means ± SEM for four pigs per dietary group.
[b]Different from control, $P < 0.001$.

notably increased by the EFA-deficient diet. This clearly indicates a lower microsomal membrane fluidity caused by EFA deficiency, especially in the C12 microenvironment.

Nonenzymatic lipid peroxidation

The main TBA-reactive compound, MDA, was significantly lower ($P < 0.05$) in EFA-deficient as compared with control pig liver microsomes (149.2 ± 9 versus 204.7 ± 15 nmol MDA/mg PL).

Calcium uptake and permeability

Ca^{++} uptake in the microsomal membrane vesicles of pigs fed the EFA-deficient diet was significantly decreased as compared with controls *(Figure 2)*. The final values, at 30 min, were 17.22 ± 0.90 and 7.58 ± 0.87 nmol Ca^{++}/mg protein for control and EFA-deficient pigs, respectively.

The efflux of Ca^{++} from microsomal vesicles loaded with calcium during the previous 6-min incubation were fitted to biexponential functions *(Figure 3)*. Ca^{++} accumulated in the microsomes was 1760 ± 60 pmoles/mg protein for controls and 990 ± 48 pmoles/mg protein for EFA-deficients. The kinetics of Ca^{++} efflux was similar in both experimental groups. The rate constants (min^{-1}) were respectively $k_1 = 1.919 \pm 0.119$, $k_2 = 0.0935 \pm 0.004$ for controls and $k'_1 = 1.649 \pm 0.0956$, $k'_2 = 0.0853 \pm 0.003$ for EFA-deficient pigs. There was no significant difference between k_1 and k'_1 or between k_2 and k'_2. Therefore, the results show that membrane permeability was not modified by the dietary treatment.

Hepatic mixed function oxidase system

As compared with controls, EFA-deficient pigs showed *(Table 6)* significant increases in microsomal cytochrome b_5 content and NADH-cytochrome b_5 reductase activity. By contrast, they had lower cytochrome P-450 content (although not significantly) and NADPH-

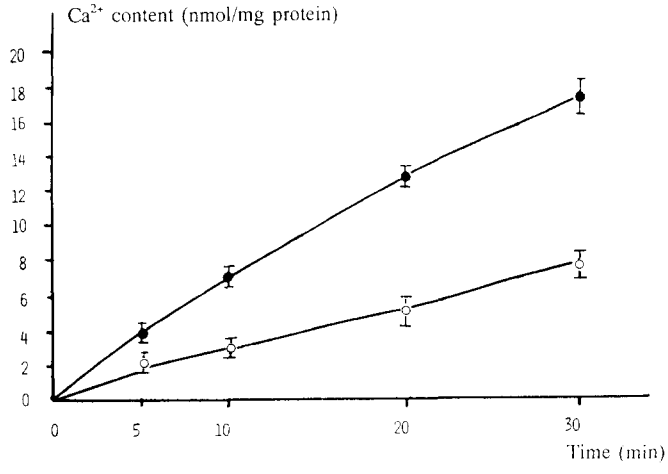

Figure 2 Time course of Ca^{2+} uptake, in the presence of oxalate, by microsomal membrane vesicles (⊢● control; ⊢○ EFA-deficient). Values are mean ± SEM for four pigs.

Table 6 Monoxigenase system in pig liver microsomal membrane[a]

	Control	EFA-deficient
Cytochrome b_5 (nmol/mg protein)	0.49 ± 0.04	0.62 ± 0.01[b]
NADH-cytochrome b_5 reductase (μmol/mg protein/30 min)	108.46 ± 12.01	163.01 ± 9.93[b]
Cytochrome P450 (nmol/mg protein)	0.55 ± 0.34	0.48 ± 0.02
NADPH-cytochrome c reductase (μmol/mg protein/30 min)	6.05 ± 0.60	3.67 ± 0.23[b]
Aniline hydroxylase (nmol/mg protein/30 min)	5.93 ± 0.71	3.67 ± 0.48[b]
Aminopyrine N-demethylase (nmol/mg protein/30 min)	38.51 ± 5.73	22.15 ± 2.64[b]
Arylhydrocarbon hydroxylase (nmol/mg protein/30 min)	5.18 ± 0.82	1.52 ± 0.25[c]

[a]Values are means ± SEM for four pigs.
[b]Significantly different from control at $P < 0.05$.
[c]Significantly different from control at $P < 0.01$.

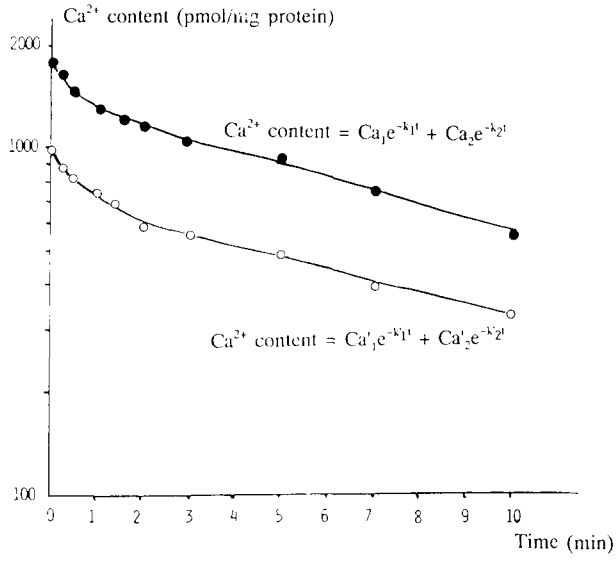

Figure 3 Passive Ca^{2+} efflux of preloaded microsomal membrane vesicles (● control; ○ EFA-deficient). Values are means for four pigs.

cytochrome c reductase activity ($P < 0.05$). Aniline hydroxylase, aminopyrine N-demethylase and benzopyrene hydroxylase activities were also significantly reduced by EFA deficiency in the pig.

Discussion

The adaptation of mammals to the dietary supply of PUFA has been shown to be achieved mostly through alterations in the lipid composition of their cellular membranes, with subsequent modifications in membrane physical properties and protein activities.[29] The present study was designed to provide a better understanding of the relationships between the effects of a dietary EFA deficiency on the membrane lipid composition and their physical properties and the observed alterations in membrane protein activities. The present results obtained in the pig clearly demonstrated that dietary EFA deficiency led to large changes in the structural and functional properties of the hepatic E.R. membrane. These diet-induced changes confirm and extend previous investigations in other animal species.[6,30]

As compared with controls, EFA-deficient pigs showed higher cholesterol levels, lower phospholipid content, and altered PL class distribution in the liver E.R. membrane. PC was shown to be located in the outer layer and the minor PL classes, mainly in the inner layer of the lipid matrix.[31] Thus, the lower proportion of membrane PC and higher PI and PS levels observed in this study could affect the membrane asymmetry and thereafter its stability. These changes may partly be explained by a decreased PC biosynthesis, as the main pathway of this synthesis has been shown to depend on membrane PUFA level.[32] Moreover, a decrease in PC biosynthesis was recently demonstrated in liver microsomes of rats fed a coconut oil-based diet as compared to a soybean oil-based diet.[33] Our results confirm the data of that work, namely a decreased 20:4 (n-6) level and n-6:n-3 ratio and an increased 22:6 (n-3) level. The increased relative amount of PI could also be considered as a contribution to preserve the membrane arachidonic acid level in EFA-deficient pig liver E.R. membrane, as this PL class is the richest in this physiologically important fatty acid.

Furthermore, according to previous studies,[1,34] PL fatty acid composition was strongly modified by EFA deficiency in the pig. Mainly unsaturated fatty acid distribution was altered, in agreement with prior observations.[6,35] Increased MUFA and 20:3 (n-9) levels and concomitant decrease in the amount of PUFA (especially those of the n-6 series) occurred in all the PL main classes. However, diet-induced alteration in long-chain PUFA distribution was particularly important. A general increase in the amount of 20:5 (n-3) and the increased PE and PS 22:6 (n-3) level could be an attempt to compensate the decreased levels of 20:4 (n-6), 22:4 (n-6), and 22:5 (n-3) in the membrane PL. In keeping with reported results on the same species,[36] the docosapentaenoic acid represented the major n-3 fatty acid in the liver E.R. membrane of control pigs (instead of DHA in rats), with a two-fold higher level than that of 22:6 (n-3). This fatty acid [20:5(n-3)] reached the same proportion as DHA in the deficient pig membranes *(Table 4)*. All of these changes were likely provided by modifications in the desaturation system. The 16:1 (n-7)/16:0 and 18:1 (n-9)/18:0 ratios, and 20:3 (n-6)/18:2 (n-6) and 20:3 (n-9)/18:1 (n-9) ratios were higher in EFA-deficient as compared to control pig membrane PL, suggesting stimulated Δ 9 and Δ 6 desaturase activities. By contrast, the lower value of 20:4 (n-6)/20:3 (n-6) ratio in hepatic E.R. membrane of the deficient animals would indicate a reduced Δ 5 desaturase activity.[1,37]

Physical properties of pig liver E.R. membrane were

affected by the nutritional treatment, a result of the above compositional alterations. Membrane fluidity was especially decreased at the 12-DS level in the EFA-deficient pig membrane as assessed by the higher value of the rotational correlation time. By contrast, it was only slightly modified at the 5-DS level, near the PL polar heads, and remained unchanged at the 16-DS level. These observations slightly differed from those previously made,[6,11] showing a decreased membrane fluidity at the 5-DS level. However, they are in strong agreement with other works[38] and also with the former,[6,11] concerning the unchanged rotational mobility in the core of the bilayer. The diet-induced lower membrane fluidity can be related to numerous alterations due to EFA deficiency, i.e., elevated Chol:PL and SM:PC ratios, decreased unsaturation index (double bond index/SFA ratio), and relative enrichment in MUFA versus PUFA; all considered as rigidifying factors for membrane bilayers.[29,39]

The large structural changes induced by the EFA-deficient diet in pig liver E.R. membrane led to important alterations of membrane functions such as calcium transport or MFO system. Ca^{++} uptake by the hepatic E.R. membrane vesicles was significantly lower in EFA-deficient than in control pigs. According to the hypothesis of Boyle et al.,[40] such a nutritional treatment induces a concomitant lower Ca^{++}-ATPase activity,[6] this enzyme being the key integral protein involved in the Ca^{++} transport across the E.R. membrane.[41] Although these protein activities have often been shown to be modulated by dietary lipids, the mechanism of this action is still not well understood. Membrane fluidity could be involved, at least partly, as suggested by numerous studies[42] and our own results. Unsaturation of fatty acyl chains of membrane PL could also be involved, although some authors have suggested that Ca^{++}-ATPase activity would be affected more by chain length than by chain unsaturation.[43] Moreover, dietary n-3 fatty acids have been shown to reduce membrane calcium transport in the sarcoplasmic reticulum. This effect has been attributed to the modifications induced in the lipid matrix structure by a lower n-6:n-3 ratio with a higher 22:6 (n-3) level.[44] The modified membrane structure in the EFA-deficient pig was also associated with a lower n-6:n-3 ratio and a higher DHA level, though only in PE and PS. However, as a lower Ca^{++} uptake was previously observed in a similar case with a decreased 22:6 (n-3) level, this factor might not be determinant and the lower n-6:n-3 ratio could by itself explain the reduced calcium transport by EFA deficiency. The present results also agree with those of Karmazyn et al.[45] in isolated myocytes, as the diet-induced change in Ca^{++} uptake was not accompanied by a modified Ca^{++} efflux from pig liver microsomes *(Figure 3)*.

Liver E.R. membrane MFO activities were severely altered by EFA deficiency. Values for control pigs lie within ranges previously reported, although arylhydrocarbon hydroxylase activity was 10-fold lower than values in rats.[8] The nutritional treatment had opposite effects on the two electron-transport chains, probably due to their different insertion into the E.R. membranes.[46] The increase in cytochrome b_5 and NADH-cyt. b_5-reductase activity has been related to the stimulation of the microsomal system of PUFA desaturation and biosynthesis.[47] The alterations in compositional data reported in the present study support these observations and are in agreement with other experiments showing lower hepatic microsomal Δ 6 and Δ 9 desaturase activities with PUFA- as compared with the SFA-enriched diet.[48] Furthermore, it has also been speculated that membrane peroxidation could interfere with desaturase activity.[49] Though we only determined the in vitro peroxidability, we showed that the latter was reduced when the dietary lipids were mostly saturated, in agreement with previous studies.[50] However, although the structural modifications triggering these alterations are not entirely clear, the present results may contribute to improving our understanding of the regulation of the fatty acid desaturation system, which is still far from being clearly understood.[51]

Cyt. P450 multienzymatic system activities were reduced by 40–70% following EFA deficiency. This result is in agreement with several reported observations in which dietary PUFA have been shown to be necessary for optimal induction of cytochrome P450[52] and to stimulate the liver microsomal MFO system;[3,53] these enzyme activities were lowered by a decreased microsomal membrane fluidity;[1] it was also demonstrated that membrane PC was needed as a functional component of this enzyme system.[54] According to these studies, we may assume that the diet-induced changes observed in the lipid composition and the physical properties of the EFA-deficient pig membrane could explain the decrease in the cytochrome P450 MFO system activities of pig hepatic E.R. membrane.

In conclusion, a 12-week dietary EFA deficiency did not influence body weight and growth rate of pigs receiving the same amount of feed, but caused dramatic changes in membrane properties. The hepatic E.R. membrane of EFA-deficient piglets showed a deeply modified lipid composition, a decreased lipid fluidity and, thereafter, altered protein activities. All the functional alterations were not produced in the same manner, suggesting a still unknown regulatory mechanism for the action of PUFA in cell membranes.

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