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# Modification of fluidity and lipid–protein relationships in pig intestinal brush-border membrane by dietary essential fatty acid deficiency

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**The effect of dietary essential fatty acid (EFA) deficiency on the dynamic molecular organization of pig intestinal brush-border membrane (BBM) was studied using purified BBM vesicles. A 6 week dietary treatment of weaning piglets induced a typical EFA-deficient pattern in the lipid composition of both plasma and epithelial membranes. In pigs fed on the EFA-deficient diet, the plasma 20:3($n-9$)/20:4($n-6$) ratio progressively increased and reached a stable value after 3 weeks of experiment, whereas it remained low (less than 0.2) in controls. In the intestinal BBM, the cholesterol/protein, phospholipid/protein and consequently the cholesterol/phospholipid ratios, as well as the phospholipid class distribution, were unchanged. In particular, the sphingomyelin/phosphatidylcholine (SM/PC) molar ratio was not affected. However, the fatty acid composition of phospholipid main classes was markedly modified, leading to decreased lipid fluidity and to a large change in membrane protein behaviour with EFA deficiency. These findings could be interpreted in terms of reduced lipid–protein interactions. Moreover, the increasing gradient of fluidity which took place within the lipidic matrix from its surface was modified by the dietary treatment, as fluidity was lowered by EFA deficiency at different depths of the layer.**

## Introduction

Dietary fats are recognized to influence lipid composition and several specific functions of cell membranes, depending on their unsaturated or saturated fatty acid content [1]. Many of the changes in the cellular functional responses following a dietary manipulation are probably caused directly by membrane lipid structural changes, which affect lipid fluidity [2,3].

It has recently been shown that EFA deficiency can modify fatty acid composition, reducing fluidity and altering enzyme activities and $Ca^{2+}$ uptake in rat liver microsomal membranes [4]. Variations in dietary triacylglycerol saturation markedly change the lipid composition and fluidity as well as the intrinsic protein activities, namely the transport properties of rat intestinal plasma membranes [5,6].

However, little is known about the mechanism of the processes involved in the above observations. Changes in the activity of certain membrane carriers, receptors and enzymes can probably be related to changes in the structure of their lipid microenvironment, leading to modified conformation or quaternary structure of the proteins embedded in the membrane [3,7].

Information regarding BBM composition and associated physical properties is essential for a full understanding of the relationship between the lipid environment and intrinsic membrane enzymatic or transport processes. As we have recently seen that fatty acid composition and lipid–protein interactions in rat BBM were influenced by the diet [8], the present experiments have been carried out in order to examine the effects of dietary EFA deficiency on brush-border membrane

Abbreviations: BBM, brush-border membrane; DPG, diphosphatidylglycerol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SM, sphingomyelin; EFA, essential fatty acid; DBI, double bond index.

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TABLE I

*Fatty acid composition of the diet*

Fatty acids 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 1% were omitted.

| Fatty acids (%, w/w) | Control | EFA-deficient |
|---|---|---|
| 8:0 | | 9.6 |
| 10:0 | | 9.5 |
| 12:0 | | 43.2 |
| 14:0 | | 13.5 |
| 16:0 | 15.0 | 9.6 |
| 18:0 | | 6.4 |
| 18:1($n-9$) | 33.3 | 3.6 |
| 18:2($n-6$) | 48.7 | 4.5 |
| 18:3($n-6$) | 1.2 | |
| 18:3($n-3$) | 0.6 | |
| 20:1($n-9$) | 0.2 | |
| Saturated | 15.0 | 91.9 |
| Monounsaturated | 33.5 | 3.6 |
| Polyunsaturated | 51.5 | 4.5 |

properties in the pig. In particular, membrane lipid composition as well as physical aspects of lipid and protein behaviour within the bilayer, were investigated.

## Materials and Methods

*Animals and diets*

Male large, white piglets were divided into two groups just after weaning (35 days old, 5.8 kg live weight). They were fed ad libitum a semipurified diet containing either 7% corn oil (control), or 7% hydrogenated coconut oil (EFA-deficient). The lipid composition of the diets is shown in Table I. After 6 weeks of dietary treatment, the animals were fasted for 18 h and killed. Blood was collected and the small intestine was rapidly removed, evected, washed with cold saline and frozen in liquid nitrogen prior to storage at −80°C. Blood was also sampled during the experimental period on weeks 1, 3, 4 and 5. In all cases it was immediately centrifuged and the plasma was stored at −80°C.

*Preparation of brush-border membrane vesicles*

The brush-border membrane vesicles were prepared from pig jejunum by an $Mg^{2+}$-precipitation method as described by Hauser et al. [9], slightly modified [10]. Aliquots of 200 µl were stored under liquid nitrogen [11] until use. The purity of BBM vesicles was routinely checked by determination of the specific activity of BBM marker enzyme sucrase [12]. The final BBM fraction showed a 14-fold enrichment in sucrase activity with respect to the original homogenate. Cross-contamination of BBM with basolateral membrane (BLM) was negligible, as shown by the 0.97-fold enrichment of the BLM marker enzyme $Na^+/K^+$-ATPase [13]. Protein was determined using the method of Lowry et al. [14] with bovine serum albumin as standard. No difference in relative BBM purity was noted between control and EFA-deficient piglets.

*Determination of the membrane lipid composition*

Total lipids were extracted according to Folch et al. [15]. Neutral lipids, glycolipids and phospholipids were separated on Sep-Pak silica cartridges (Waters, Milford, MA) by successive elution with chloroform, acetone and methanol [16,17]. Cholesterol was assessed from the neutral lipid fraction by an enzymatic method [18]. Phospholipids were separated by HPLC, following the method of Geurts van Kessel et al. [19]. A Beckman 332 gradient liquid chromatograph (Beckman, Gagny, France) equipped with a LKB detector (Uvicord 2158 SD) set at 206 nm was used. The separation of phospholipid classes was achieved by an Ultrasphere-Si column (10 × 250 mm) from Beckman at a flow rate of 2.5 ml/min. The chromatographic system was programmed for gradient elution using mobile phases: solvent A, *n*-hexane/2-propanol/water (6 : 8 : 0.75, v/v) and solvent B, *n*-hexane/2-propanol/water (6 : 8 : 1.4, v/v). A linear gradient of solvent B in solvent A was formed from 0 to 100% in 10 min, starting 10 min after the beginning of the elution. At 60 min, solvent A concentration was increased up to 100% in 5 min. Under these conditions, individual phospholipids were all separated within 75 min. The purity of each phospholipid class was determined by TLC according to Gilfillan et al. [20] and quantitative estimation of phospholipids was done by determination of inorganic phosphorus ($P_i$) using the Bartlett procedure [21].

The fatty acid composition of phospholipids was determined after transmethylation [22]. Fatty acid methyl esters were analysed using a Packard model 427 gas chromatograph (Rungis, France) equipped with a flame ionization detector and a CP Wax 52 CB bonded fused silica capillary column (50 m × 0.2 mm internal diameter). Hydrogen was the carrier gas, with a flow rate of 1–2 ml/min. The detector and the injector were set at 300 and 260°C, respectively, and the oven temperature programmed from 140 to 225°C at a heating rate of 6 C°/min. The identification of peaks was made by comparison of equivalent chain lengths (ECL) with those of authentic fatty acid methyl esters. Peak areas were determined by a Delsi (Enica 10) integrator (Delsi, Suresnes, France). Automatic expression of peak areas as weight percentage in the fatty acid mixture and ECL were obtained with a fitted microcomputer.

*Spin-labeling*

Membrane lipids were probed with 5-, 7-, 10-, 12- and 16-nitroxide stearic acids (5-, 7-, 10-, 12- and 16-NS)

with about 100 μl of BBM vesicles (18 mg of protein/ml) which were incubated with 1 μl of 10 mM stearic acid in dimethylsulphoxide for 5–10 min. Then, every solution was washed with 1 ml PBS buffer (pH 7.4) and centrifuged at 48000 × *g* for 15 min. The first pellet was redispersed in 100 μl buffer and kept in ice before analysis by ESR.

For protein labeling, BBM vesicles (150 μl) of control and EFA-deficient pigs, with approximately the same protein level (18 mg/ml), were incubated in the same volume of buffer (PBS) (pH 7.4) with maleimido-Tempo [23]. After shaking, the suspended vesicles were kept at 4°C overnight. Then, membranes were diluted in 3 ml of buffer and centrifuged at 48000 × *g* for 15 min to remove unreacted spin-label. Pellets were suspended in 100 μl buffer and kept in ice before analysis by ESR.

*ESR measurements*

ESR spectra were recorded at 9.14 GHz with an E-109 Varian-X Band spectrometer. The temperature was monitored with a thermocouple and measurements were made at increments of 2 C° between 0 and 40°C. The microwave power was maintained at 40 mW and the magnetic field sweep was of 100 G. For spin labels undergoing anisotropic motion (5-, 7-, 10- and 12-NS), the $2A_{\parallel}$ parameter was measured between the outer hyperfine peaks of the spectrum. The $2A_{\parallel}$ parameter gave values similar to those of the order parameter, *S*, involving inner and outer hyperfine peaks. For 16-doxylstearic acid undergoing rapid and essentially isotropic motion, the rotational correlation frequency, $\nu$, of the magnetic probe was calculated with the equation:

$$\nu = 2.1^8/\Delta h_0\left(\sqrt{h_0/h_1 - 1}\right)$$

with $h_0$ the height of the central peak, $h_1$ the height of the peak at the lowest magnetic field, $\Delta h_0$ the width of the central peak.

The *w/s* parameter was used for maleimido-Tempo, *w* and *s* measurements corresponding to the amplitudes of the weakly and strongly immobilized spin-labels, respectively [23].

## Chemicals

All reagents were of analytical grade. The following spin-labels were purchased from Molecular Probes (Eugene, OR): stearic acids with the 4-4-dimethyl-3-oxazolidinyloxy group (doxyl group) attached to either carbon atom 5 (5-doxylstearic acid or 5-NS), 7 (7-doxylstearic acid or 7-NS), 10 (10-doxylstearic acid or 10-NS), 12 (12-doxylstearic acid or 12-NS), 16 (16-doxylstearic acid or 16-NS) and maleimido-Tempo (3-maleimido-2,2′,5,5′-tetramethyl-1-pyrrolidinyloxyl).

## Results

*Lipid composition of plasma phospholipids and BBM vesicles*

The fatty acid composition of plasma phospholipids was greatly modified by EFA deficiency. In particular, linoleic and arachidonic acid levels were markedly depressed (Fig. 1), whereas the 5,8,11-eicosatrienoic acid (20 : 3($n$ − 9)) level (as well as the 20 : 3($n$ − 9)/20 : 4($n$ − 6) ratio) increased from the 3rd week and then remained constant until the end of the experiment.

In the intestinal BBM, with respect to protein, the amount of cholesterol and phospholipids as well as the molar ratio of cholesterol to phospholipids were unchanged by EFA deficiency (Table II). The phospholipid class distribution and, in particular, the sphingomyelin/phosphatidylcholine (SM/PC) molar ratio, was also not different in the two groups. The average percentages were as follows: PC: 33.9 ± 1.8; PE: 34.6 ± 1.1; PS: 9.7 ± 1.1; SM: 8.5 ± 1.0; PI: 7.2 ± 1.0; LPC: 3.2 ± 0.6; DPG: 1.3 ± 0.3; LPE: 1.5 ± 0.4 for 100% total phospholipids corresponding to 353 ± 82 nmol/mg of protein (EFA-deficient) and 403 ± 12 nmol/mg of protein (control), respectively. However, there were many changes in the fatty acid composition of BBM phospholipid main classes (Tables IIIA and

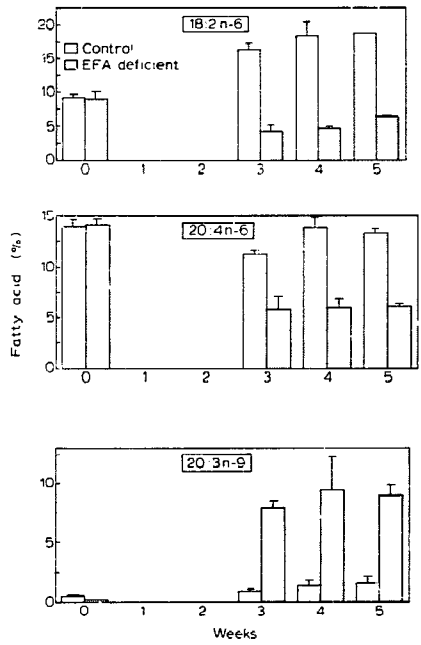


Fig. 1. Changes in linoleic, arachidonic and eicosatrienoic acid levels in plasma phospholipids of EFA-deficient (■) and control (□) piglets. Data represent means ± S.E. for three determinations.

TABLE II

*Effect of EFA deficiency on phospholipid and cholesterol contents of pig intestinal BBM vesicles*

Data are means ± S.E. for three membrane preparations.

| | Cholesterol/ protein (w/w) | Phospholipid/ protein (w/w) | Cholesterol/ phospholipid (mol/mol) |
|---|---|---|---|
| Control | 0.10 ± 0.01 | 0.34 ± 0.01 | 0.65 ± 0.05 |
| EFA-deficient | 0.08 ± 0.00 | 0.29 ± 0.07 | 0.62 ± 0.08 |

IIIB) of EFA-deficient pigs as compared to controls. Specific distribution of fatty acids within the different phospholipid classes was altered by the dietary treatment. A large decrease in linoleic acid (18 : 2)($n-6$) occurred in all membrane phospholipids, except the SM class, in which it was already very low and remained unchanged. In contrast, the 5,8,11-eicosatrienoic acid level was increased in all the phospholipid classes, as well as those of oleic (18 : 1($n-9$)) and palmitoleic (16 : 1($n-7$)) acids, especially in the two main classes (PC and PE). However, the arachidonic acid (20 : 4($n-6$)) level was unchanged in most of the phospholipid classes. The saturated fatty acid level was not significantly affected, but the total amount of monounsaturated fatty acids was higher in EFA-deficient pig BBM. In contrast, the total $n-6$ fatty acids decreased so that the PUFA/SFA ratio was markedly lower in the deficient animal membranes. Moreover, the double bond index (DBI) to saturated fatty acid ratio was unchanged, while 16 : 1($n-7$)/16 : 0, 18 : 1($n-9$)/18 : 0 and 20 : 4($n-6$)/18 : 2($n-6$) ratios were higher with EFA deficiency.

*Spin-labeling*

Figs. 2, 3 and 4 illustrate the results with the lipid spin-labels. The former clearly shows that, whatever the experimental temperature, the $2A_{\parallel}$ parameter was

TABLE IIIA

*Fatty acid composition of phospholipid main classes of pig BBM major classes*

Fatty acids are designated as in Table I values(mol%) are means ± S.E. for three membrane preparations per dietary group. DBI: double bond index = Σ(mol% each unsaturated fatty acid × number of double bonds of the same fatty acid); SFA: saturated fatty acids; DBI/SFA: unsaturation index; PUFA: polyunsaturated fatty acids.

| Fatty acid | PC | | PE | |
|---|---|---|---|---|
| | control | EFA-deficient | control | EFA-deficient |
| 14 : 0 | 0.60 ± 0.26 | 2.96 ± 2.02 | 0.49 ± 0.10 | 2.27 ± 0.11 |
| 16 : 0 | 35.28 ± 0.63 | 29.83 ± 4.06 | 10.62 ± 0.11 | 10.71 ± 0.99 |
| 16 : 1 ($n-9$) | | 1.77 ± 0.40 | 0.45 ± 0.08 | |
| 16 : 1 ($n-7$) | 1.67 ± 0.31 | 3.92 ± 0.48 | 0.58 ± 0.23 | 7.78 ± 1.05 |
| 18 : 0 | 10.96 ± 1.28 | 12.27 ± 1.50 | 20.55 ± 3.05 | 17.34 ± 2.72 |
| 18 : 1 ($n-9$) | 15.87 ± 3.60 | 28.84 ± 4.89 | 17.90 ± 1.79 | 28.08 ± 0.90 |
| 18 : 1 ($n-7$) | 0.91 ± 0.06 | 3.58 ± 0.95 | 2.11 ± 0.22 | 2.06 ± 0.03 |
| 18 : 2 ($n-6$) | 27.40 ± 2.01 | 8.08 ± 2.32 | 26.30 ± 0.57 | 8.03 ± 2.00 |
| 18 : 3 ($n-3$) | 0.18 ± 0.08 | 1.17 ± 0.05 | 0.62 ± 0.26 | 1.79 ± 0.41 |
| 20 : 0 | 0.29 ± 0.10 | 0.49 ± 0.02 | 0.31 ± 0.09 | 0.42 ± 0.11 |
| 20 : 1 ($n-9$) | 0.21 ± 0.08 | 0.22 ± 0.04 | 0.24 ± 0.01 | 0.29 ± 0.01 |
| 20 : 2 ($n-6$) | | | 0.93 ± 0.33 | |
| 20 : 3 ($n-9$) | | 1.59 ± 0.44 | 0.06 ± 0.00 | 4.26 ± 0.41 |
| 20 : 3 ($n-1$) | 0.26 ± 0.02 | 0.20 ± 0.07 | 0.41 ± 0.05 | 0.53 ± 0.04 |
| 20 : 4 ($n-6$) | 3.28 ± 0.06 | 3.97 ± 0.91 | 10.03 ± 0.37 | 10.55 ± 0.49 |
| 20 : 5 ($n-3$) | 0.15 ± 0.05 | 0.34 ± 0.01 | 0.42 ± 0.24 | 0.75 ± 0.06 |
| 22 : 0 | 0.32 ± 0.02 | 0.41 ± 0.16 | 0.25 ± 0.06 | 0.25 ± 0.06 |
| 22 : 1 ($n-9$) | 0.11 ± 0.05 | 0.10 ± 0.01 | 0.16 ± 0.05 | 0.37 ± 0.11 |
| 22 : 4 ($n-6$) | 0.52 ± 0.10 | 0.60 ± 0.11 | 3.69 ± 0.48 | 1.53 ± 0.27 |
| 22 : 5 ($n-6$) | 0.54 ± 0.02 | 1.31 ± 0.80 | 1.64 ± 0.49 | 2.25 ± 0.69 |
| 22 : 5 ($n-3$) | 0.21 ± 0.00 | 0.55 ± 0.10 | 1.26 ± 0.00 | 1.36 ± 0.11 |
| 22 : 6 ($n-3$) | 0.42 ± 0.06 | 1.03 ± 0.17 | 0.37 ± 0.12 | 0.37 ± 0.10 |
| 24 : 0 | 0.38 ± 0.05 | 0.92 ± 0.15 | 0.34 ± 0.11 | 0.42 ± 0.03 |
| 24 : 1 ($n-9$) | 0.71 ± 0.01 | 0.88 ± 0.15 | 0.35 ± 0.04 | 0.21 ± 0.01 |
| 20 : 3/20 : 4 | 0.00 ± 0.00 | 0.39 ± 0.02 | 0.01 ± 0.00 | 0.41 ± 0.06 |
| DBI | 141.58 ± 0.13 | 132.14 ± 6.98 | 181.99 ± 2.22 | 163.89 ± 5.47 |
| DBI/SFA | 2.96 ± 0.09 | 2.90 ± 0.42 | 5.64 ± 0.55 | 5.42 ± 0.35 |
| PUFA/SFA | 0.69 ± 0.01 | 0.40 ± 0.09 | 1.42 ± 0.15 | 1.02 ± 0.07 |
| Σ($n-6$) | 32.34 ± 1.66 | 13.96 ± 2.71 | 43.03 ± 0.16 | 22.89 ± 1.00 |
| Σ($n-3$) | 0.86 ± 0.19 | 2.97 ± 0.10 | 2.67 ± 0.62 | 3.67 ± 0.73 |
| Σ($n-9$)+($n-7$) | 18.98 ± 2.95 | 35.84 ± 5.41 | 21.44 ± 1.70 | 42.63 ± 1.65 |

TABLE IIIB

*Fatty acid composition of phospholipid main classes of pig BBM minor classes*

| Fatty acid | PS | | PI | | SM | |
|---|---|---|---|---|---|---|
| | control | EFA-deficient | control | EFA-deficient | control | EFA-deficient |
| 14:0 | 1.49± 0.12 | 3.46±2.21 | 2.69±0.81 | 1.32±0.12 | 3.82±3.00 | 5.18±0.42 |
| 16:0 | 15.92± 1.03 | 9.81±1.27 | 16.64±0.60 | 15.49±2.75 | 69.38±7.57 | 43.77±0.82 |
| 16:1 (*n*−9) | 2.91± 0.05 | 1.38±0.99 | 5.12±1.78 | 2.93±1.05 | 0.61±0.20 | |
| 16:1 (*n*−7) | 5.13± 1.82 | 2.31±0.28 | 4.25±1.63 | 1.80±0.07 | 2.34±0.03 | 5.39±1.82 |
| 18:0 | 35.76± 3.31 | 35.41±3.39 | 23.08±1.11 | 21.85±1.90 | 10.03±3.97 | 18.87±1.54 |
| 18:1 (*n*−9) | 15.11± 5.57 | 24.55±3.35 | 12.86±0.50 | 22.82±1.17 | 4.75±2.75 | 15.47±1.65 |
| 18:1 (*n*−7) | 0.64± 0.03 | 1.11±0.08 | 0.84±0.00 | | 0.20±0.08 | |
| 18:2 (*n*−6) | 13.41± 0.14 | 7.30±1.85 | 18.56±6.14 | 5.80±2.19 | 2.16±1.52 | 3.56±1.80 |
| 18:3 (*n*−3) | 2.26± 1.88 | 1.46±0.51 | 12.77±0.99 | 1.57±0.07 | 0.54±0.06 | 0.60±0.08 |
| 20:0 | 1.27± 0.76 | 0.68±0.13 | 0.75±0.01 | 0.48±0.20 | 0.49±0.37 | 1.50±0.42 |
| 20:1 (*n*−9) | 2.41± 0.95 | 0.25±0.06 | | | | 0.25±0.09 |
| 20:2 (*n*−6) | | | 0.46±0.13 | | 0.27±0.10 | |
| 20:3 (*n*−9) | | 1.15±0.26 | | 4.58±1.53 | 0.18±0.05 | 0.59±0.20 |
| 20:3 (*n*−6) | 2.42± 2.21 | 0.36±0.10 | 0.59±0.02 | 0.78±0.30 | 0.15±0.03 | 0.32±0.07 |
| 20:4 (*n*−6) | 1.98± 0.51 | 4.05±0.80 | 9.73±0.32 | 11.07±0.01 | 3.69±0.51 | 2.74±0.55 |
| 20:5 (*n*−3) | 0.86± 0.13 | 0.29±0.05 | | 1.07±0.36 | | 0.43±0.05 |
| 22:0 | 1.48± 0.61 | 0.59±0.14 | | | 0.31±0.09 | 0.39±0.07 |
| 22:1 (*n*−9) | 0.29± 0.04 | 0.43±0.22 | | | 2.51±0.81 | 0.50±0.05 |
| 22:4 (*n*−6) | 0.82± 0.19 | 0.78±0.09 | 1.15±0.06 | 1.11±0.21 | 0.13±0.06 | 0.24±0.04 |
| 22:5 (*n*−6) | 4.39± 2.40 | 1.50±1.05 | 2.06±1.18 | 4.21±0.33 | | |
| 22:5 (*n*−3) | 1.35± 1.03 | 1.39±0.19 | 0.54±0.11 | 1.21±0.48 | | |
| 22:6 (*n*−3) | 1.32± 0.74 | 0.71±0.15 | 0.46±0.21 | 0.99±0.17 | | |
| 24:0 | 1.18± 0.66 | 0.63±0.13 | 0.41±0.19 | 0.88±0.15 | 0.31±0.06 | 0.31±0.05 |
| 24:1 (*n*−9) | 1.19± 0.22 | 0.26±0.08 | 0.13±0.09 | 0.17±0.02 | 0.45±0.04 | 0.39±0.06 |
| 20:3/20:4 | 0.00± 0.00 | 0.28±0.03 | 0.00±0.00 | 0.41±0.14 | 0.02±0.02 | 0.24±0.12 |
| DBI | 155.92±11.04 | 135.19±3.63 | 159.07±0.97 | 178.07±5.43 | 107.63±7.19 | 104.73±4.72 |
| DBI/SFA | 3.33± 0.84 | 2.69±0.16 | 3.69±0.24 | 4.40±0.26 | 1.29±0.18 | 1.50±0.04 |
| PUFA/SFA | 0.63± 0.21 | 0.37±0.02 | 0.82±0.18 | 0.77±0.11 | 0.08±0.04 | 0.12±0.03 |
| Σ(*n*−6) | 23.73± 0.46 | 13.99±2.10 | 32.25±6.16 | 22.96±3.04 | 6.12±2.17 | 6.74±2.30 |
| Σ(*n*−3) | 5.36± 4.07 | 3.75±0.49 | 2.77±1.08 | 3.47±0.97 | 0.27±0.27 | 0.81±0.13 |
| Σ(*n*−9)+(*n*−7) | 21.54± 4.79 | 30.61±4.14 | 20.57±1.37 | 32.30±0.64 | 9.37±3.99 | 22.40±3.33 |

higher in EFA-deficient than in control pig intestinal BBM. This means that EFA deficiency causes an increased membrane organization near the polar head (5-NS and 7-NS labels) as well as deeper in the membrane phospholipid leaflet (10-NS and 12-NS labels). Furthermore, the difference in the $2A_{\parallel}$ values became greater as the position of the nitroxide probe was deeper in the bilayer, at least until the 12th carbon atom of the acyl chains (Fig. 3). In contrast, the rotational correlation parameter measured for the 16-NS label was not modified by the dietary treatment, as shown in Fig. 4.

EFA deficiency led to a higher *w/s* ratio whatever the temperature (Fig. 5). In addition, variations in the *w/s* ratio with respect to temperature were similar for both dietary groups. Therefore, the variation in the difference between the *w/s* ratios measured on EFA-deficient and control pig BBM was also similar, both slightly increasing with temperature. The increase in the weakly immobilized label of the EFA-deficient pig membrane was more rapid as the temperature increased.

## Discussion

Increasing attention has been paid in recent years to the interactions between membrane lipids and proteins, with the aim of better understanding membrane function [24–26]. The present study shows that the structural organization of lipids and proteins of pig enterocyte luminal membrane is markedly altered by an inadequate dietary content of essential polyunsaturated fatty acids.

Lower fluidity of rat intestinal membranes following an EFA-deficient dietary treatment has been shown to be related mainly to a higher cholesterol/phospholipid molar ratio than in the control animal membranes [5,27]. However, perhaps due to the lower lipid content of our experimental diet (7 vs. 37% in other experiments [5]), no variations were observed in either cholesterol or phospholipid levels relative to proteins as well as in the phospholipid class distribution of porcine BBM. The cholesterol/phospholipid as well as the SM/PC molar ratios were not affected by the dietary treatment and

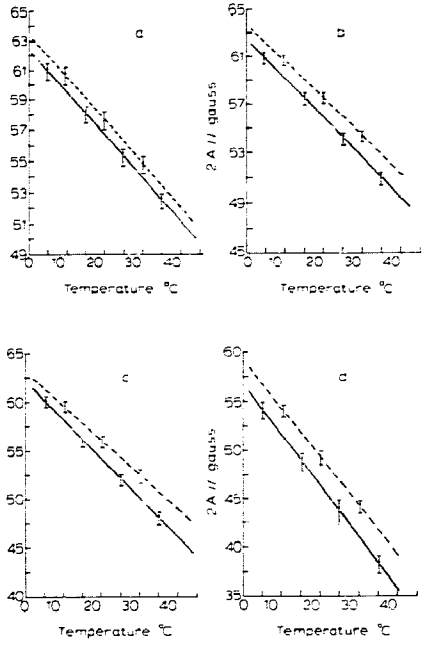


Fig. 2. Temperature variations of $2A_{\parallel}$ parameter measured with (a) 5-NS (b) 7-NS (c) 10-NS and (d) 12-NS spin-labels in intestinal BBM vesicles of EFA-deficient (— — —) and control (———) pigs. Data represent means ± 2 S.E. for three experiments.

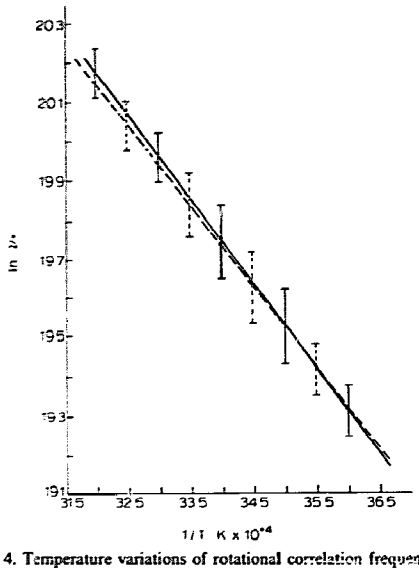


Fig. 4. Temperature variations of rotational correlation frequency at low field of 16-NS spin-label in intestinal BBM vesicles of EFA-deficient (— — —) and control (———) pigs. Data represent means ± 2 S.E. for three experiments.

therefore cannot be used to explain the changes in the membrane physical properties of EFA-deficient piglets. These changes can thus very likely only be imputed to the modifications induced in the fatty acid composition of BBM phospholipids by EFA deficiency. As the unsaturation index is not affected by the dietary treatment, the major change therefore concerns the individual variations of fatty acids.

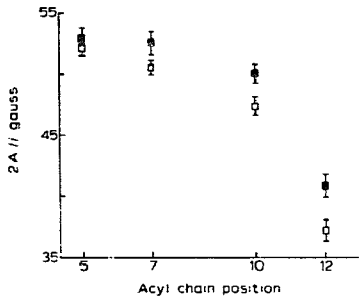


Fig. 3. Variation of $2A_{\parallel}$ parameter with the position of the doxyl group attached to the spin-label stearyl chain in the intestinal BBM vesicles of EFA-deficient (■) and control (□) pigs. Data represent means ± 2 S.E. for three determinations at 37 °C.

In the EFA-deficient pig membrane, the dramatic decrease in linoleic acid and other acids of the $(n-6)$ series was quantitatively compensated by higher levels

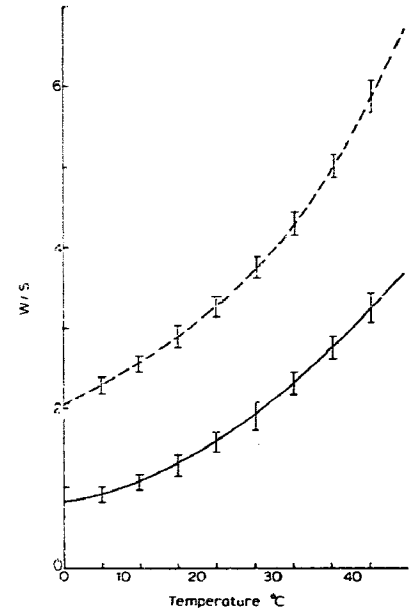


Fig. 5. Temperatre variations of the $w/s$ ratio measured on EFA-deficiency (— — —) and control (———) pig BBM vesicles labeled on their proteins. Data represent means ± 2 S.E. for four determinations.

of oleic and palmitoleic acids and, although to a lesser extent, by the synthesis of 5,8,11-eicosatrienoic acid. This physiological response to EFA deficiency, like the increase in the $20:3(n-9)/20:4(n-6)$ ratio [28,29], partially reflects the diet composition but also results from the stimulation of $\Delta^9$ and $\Delta^6$ desaturases, as witnessed by the higher $16:1(n-7)/16:0$, $18:1\ (n-9)/18:0$ and $20:4(n-6)/18:2(n-6)$ ratios in most of the phospholipid classes. This leads to the nonsignificant change observed in the DBI/SFA ratio, but to a lower PUFA/SFA ratio in EFA-deficient pig BBM, as the level of SFA remained unchanged, while that of PUFA markedly decreased. Moreover, contrasting with the former, a BBI calculated only on PUFA ($DBI_{PUFA}$) is modified with the diet and thereafter the $DBI_{PUFA}$/SFA ratio is reduced by EFA deficiency. Physiological processes probably take place in the EFA-deficient animal membrane in an attempt to compensate the lower level of PUFA, even if the level of $20:4(n-6)$ has been preserved. These involve, for example, the general appearance of eicosatrienoic acid in the phospholipids and perhaps also the trend of an increasing level of $(n-3)$ PUFA (up to 245% in PC). In particular, according to the well-known conformation of bilayer phospholipids and especially the parallel stacking of hydrocarbon chains [30], it can be assumed that the different nature of unsaturated fatty acids in EFA-deficient and control pig BBM leads to different distribution of double bonds in the depth of the layer. This structural change could induce significant modification in the physicochemical properties of the membranes.

The classical increase in membrane disorder with the increasing membrane depth [31] was found again here. However, the order profile gradient was modified in EFA-deficient as compared to control pig BBM. As strongly suggested by Fig. 3, membrane orientational order was always greater in the former. Nevertheless, it can be noticed that results with 16-NS measurements show that the rotational mobility was not affected at this depth by EFA deficiency. The increased order in the EFA-deficient pig membrane lipids at different depths of the layer is probably due to higher interactions between the acyl chains of the phospholipids than in the control animal BBM. This could well be related to the depressed level of PUFA and the concomitant higher saturated and monounsaturated fatty acid levels. This leads to a higher degree of rigidification, due to a packing effect of saturated acyl chains via magnified Van der Waals forces within the EFA-deficient pig membrane [31]. Moreover, we also observed an increased $w/s$ ratio in the EFA-deficient pig BBM. Assessment of the amplitudes of $w$ and $s$ peaks showed an enhanced amount of the weakly immobilized label in the EFA-deficient pig membranes as compared to those of the controls, with no change in the strongly immobilized label. Classically, the maleimide spin-label is used in analysis of structural changes around SH groups of biological membrane proteins. However, the reporter group of the spin label can be immobilized not only by the nature of the tridimensional structure of the binding site, but also by intra- and intermolecular protein folding [32]. As the temperature-dependence of the $w/s$ ratio is the same in both EFA-deficient and control pig BBM, protein conformational change [33] very likely does not occur in this temperature range. This is the mere fact we can conclude from the $w/s$ experiments. A more complete explanation of this result, referring for instance to relationships with either protein glycosylation or processing, new distribution of individual proteins or vertical displacement of some intrinsic proteins [34–36] needs further investigation Nevertheless, the increased level of weakly immobilized labels in EFA-deficient pig membrane could mean a decrease in lipid–protein interactions. Therefore, this could be related to changes in functional properties of membrane proteins, as found in such cases [37–39] of modified fatty acid composition.

Our results point out that EFA deficiency leads to a modified membrane lipid matrix. This observation could be related to both the increased interactions between the acyl chains and the different pattern of unsaturation in the EFA-deficient pig membrane. This could be responsible for the loss of some interactions between membrane constituents which are needed to maintain the normally active membrane protein structure. It seems that a higher cohesion of the lipid matrix leads here to a lower cohesion between lipids and some proteins. Thus, it could justifiably be speculated that the increased level of $(n-9)$ and $(n-7)$, as well as the loss of $(n-6)$ fatty acids, provided by EFA deficiency could interfere in protein function. Undoubtedly, these phenomena belong to the regulatory mechanisms which remain to be clearly characterized but which very probably involve still unknown EFA properties in the membrane [40].

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## References

1 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
2 Brenner, R.R. (1984) Prog. Lipid Res. 23, 69–96.
3 Spector, A.A. and Yorek, M.A. (1985) J. Lipid Res. 26, 1015–1035.
4 Christon, R., Fernandez, Y., Cambon-Gros, C., Périquet, A.,

Deltour, P., Léger, C.L. and Mitjavila, S. (1988) J. Nutr. 118, 1311–1318.
5 Brasitus, T.A., Davidson, N.O. and Schachter, D. (1985) Biochim. Biophys. Acta 812, 466–472.
6 Thompson, A.B.R. Keelan, M., Clandinin, M.T. and Walker, K. (1986) J. Clin. Invest. 77, 279–288.
7 Salesse, R., Garnier, J., Leterrier, F., Daveloose, D. and Viret, J. (1982) Biochemistry 21, 1581–1586.
8 Christon, R., Even, V., Daveloose, D., Viret, J., Linard, A., Thévenoux, J. and Léger, C.L. (1988) Reprod. Nutr. Dév. 28, 657–658.
9 Hauser, H., Howell, K., Dawson, R.M.C. and Bowyer, D.E. (1980) Biochim. Biophys. Acta 602, 567–577.
10 Brot-Laroche, E. and Alvarado, F. (1984) Biochim. Biophys. Acta 775, 175–181.
11 Stevens, B.R., Wright, S.H., Hirayama, B.S., Gunther, R.D., Ross, H.J., Harms, V., Nord, E., Kippen, I. and Wright, E.M. (1982) Membr. Biochem. 4, 271–282.
12 Dahlquist, A. (1968) Anal. Biochem. 22, 99–107.
13 Sha'afi, R.I., Naccache, P., Raible, D., Krepcio, A., Showell, H. and Becker, E.L. (1976) Biochim. Biophys. Acta 448, 638–641.
14 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
15 Folch, J., Lees, M. and Sloane-Stanley, G.H. (1957) J. Biol. Chem. 226, 497–509.
16 Rouser, G., Kritchevsky, G., Simon, G. and Nelson, G.J. (1967) Lipids 2, 37–40.
17 Juaneda, P. and Rocquelin, G. (1985) Lipids 20, 40–41.
18 Chemin Douaud, S. and Karleskind, A. (1979) Rev. Fr. Corps Gras 26, 313–316.
19 Geurts Van Kessel, W.S.M., Hax, W.M.A., Demel, R.A. and De Gier, J. (1977) Biochim. Biophys. Acta 486, 524–530.
20 Gilfillan, A.M., Chu, A.J., Smart, D.A. and Rooney, S.A. (1983) J. Lipid Res. 24, 1651–1656.
21 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468.
22 Berry, J.F., Cevellos, W.H. and Wade, R.R. (1965) J. Am. Oil Chem. Soc. 42, 492–500.
23 Butterfield, D.A., Oeswein, J.Q. and Markesbery, W.R. (1977) Nature 267, 453–455.
24 Benga, G. (1985) in Structure and Properties of Cell Membranes, Vol. 1 (Benga, G., ed.), pp. 159–188, CRC Press, Boca Raton, FL.
25 Carruthers, A. and Melchior, D.L. (1986) Trends Biochem. Sci. 11, 331–335.
26 Lenaz, G. (1987) Biosci. Rep. 7, 823–837.
27 Schachter, D. (1985) in Progress in Protein–Lipid Interactions (Watts, A. and De Pont, J.J.H.H.M., eds.), Vol. 1, pp. 231–258, Elsevier, Amsterdam.
28 Holman, R.T. (1971) Prog. Chem. Fats Other Lipids 9, 275–348.
29 Rivers, J.P.W. and Frankel, T.L. (1981) Br. Med. Bull. 37, 59–64.
30 Hauser, H., Pascher, I., Pearson, R.H. and Sundell, S. (1981) Biochim. Biophys. Acta 650, 21–51.
31 Kleinfeld, A.M. (1987) in Current Topics in Membranes and Transport, Vol. 29 (Bronner, F., ed.), pp. 1–27, Academic Press, London.
32 Gaczynska, M. and Bartosz, G. (1987) Studia Biophys. 120, 155–161.
33 Ohyashiki, T., Taka, M. and Mohri, T. (1985) J. Biol. Chem. 260, 6857–6864.
34 Amar, A., Rottem, S. and Razin, S. (1979) Biochim. Biophys. Acta 552, 457–467.
35 Shinitzky, M. (1984) in Physiology of Membrane Fluidity, Vol. 1 (Shinitzky, M., ed.), pp. 1–51, CRC Press, Boca Raton, FL.
36 Viret, J., Dormont, D., Molle, D., Court, L., Leterrier, F., Cathala, F., Gibbs, C.J. Jr. and Gajdusek, D.C. (1981) Biochem. Biophys. Res. Commun. 101, 830–836.
37 Castuma, C.E. and Brenner, R.R. (1983) Biochim. Biophys. Acta 729, 9–16.
38 Neelands, P.J. and Clandinin, M.T. (1983) Biochem. J. 212, 573–583.
39 Murakami, M., Takada, K., Fujii, T. and Muranishi, S. (1988) Biochim. Biophys. Acta 939, 238–246.
40 Léger, C., Frémont, L., Alessandri, J-M., Christon, R. and Linard, A. (1987) Can. Nutr. Diét. 12, 1–9.