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# Phospholipid hydroperoxide glutathione peroxidase activity and vitamin E level in the liver microsomal membrane: effects of age and dietary $\alpha$-linolenic acid deficiency

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**Abstract**

Age and diet-induced variations of phospholipid hydroperoxide glutathione peroxidase (PHGPx) activity and $\alpha$-tocopherol concentration in the liver microsomal membrane were studied in male Wistar rats fed a semipurified diet either balanced in n-6 and n-3 polyunsaturated fatty acids (PUFA) (Control) or deprived of $\alpha$-linolenic acid, i.e. n-3 PUFA (Deficient) over two generations. The animals were studied at the age of 6 months (adult) or 24 months (old). Both PHGPx activity and vitamin E level were significantly higher in 24-month old rats as compared to 6-month old rats. By contrast, the thiobarbituric acid reactive substances (TBARS) following stimulated in vitro peroxidation of membrane lipids were markedly lower ($P < 0.01$) with aging. The fatty acid composition of microsomal membrane phospholipids (PL) was also considerably modified by age. In particular, the levels of arachidonic acid and total n-6 PUFA were lower ($P < 0.001$) whereas n-3 PUFA levels were higher ($P < 0.001$) in most PL main classes. The $\alpha$-linolenic acid deficiency markedly influenced these age-related changes. The higher PHGPx activity in the old rats as compared to the adult rats was only significant in those fed the control diet. In the 6-month old rats (but not in the 24-month old rats), the deficient diet led to a higher membrane vitamin E level and to lower TBARS production than the control diet. The results suggest that the nature of dietary PUFA may influence the age-related variations in this pair of membrane antioxidants and also in the fatty acid composition of microsomes. 

*Keywords:* Phospholipid hydroperoxide glutathione peroxidase; Vitamine E; Membrane; Aging; N-3 fatty acids

## 1. Introduction

The aging process has often been reported to be associated with decreased functional efficiency in the antioxidant defense system [1]. The latter mainly consists of free radical scavengers such as glutathione peroxidases (GPx) and vitamin C and vitamin E. In the GPx super-family, the selenoenzyme phospholipid hydroperoxide glutathione peroxidase (PHGPx, EC 1.11.1.12) is present in several mammalian tissues, in both free and membrane-bound form. It utilizes thiols such as glutathione to specifically scavenge lipid hydroperoxides. In fact, it reduces the hydroperoxides of phospholipid, cholesterol and cholesteryl ester in biomembranes and therefore specifically protects membrane phospholipids against peroxidation [2,3]. PHGPx also reduces hydroperoxides from oxidized lipoproteins and therefore plays a major role in the antioxydative defense system [4]. Furthermore, the enzyme may also be involved in certain other functions, such as spermatogenesis, being in testes Leydig cells [5], or as a modulator of leukotriene production [6] and an inhibitor of lipoxygenase [7].

Moreover, vitamin E is known to be one of the most active natural antioxidant in cell membranes [8], by protecting polyunsaturated fatty acids (PUFA) against oxidation or against the effect of endogenous phospholipases and by maintaining membrane integrity and permeability [9]. The antioxidant effect of vitamin E is combined with that of Se-GPx in regulating eicosanoid metabolism [10], and a synergic relationship between PHGPx activity and membrane vitamin E content has also been demonstrated [7,11].

Dietary fats have been shown to affect cellular anti-

Abbreviations: DBI, double bond index; GPx, glutathione peroxidase; MDA, malonaldehyde; MUFA, monounsaturated fatty acids; PHGPx, phospholipid hydroperoxide glutathione peroxidase; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TBARS, thiobarbituric acid reactive substances.

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oxidants. Above all, the effects of the nature of dietary PUFA and particularly that of fish oil fatty acids on the antioxidant enzyme activities and in particular GPx activity have been demonstrated [12,13]. However, only a few studies have been carried out on the relationships between dietary fatty acids and PHGPX activity. In particular, no significant differences between the effects of dietary fish oil and coconut oil were found in PHGPx activity while that of GPx was decreased by fish oil enriched diet [14]. Moreover, the latter led to a significant increase in lipid peroxidation products and a decrease in vitamin E concentration. Furthermore, the effect of dietary PUFA on either plasma or liver vitamin E concentration has been shown to be different [13].

As compared to adult animals, young rats have been shown to exhibit lower PHGPx together with GPx activities while old rats had an unchanged or higher (not significantly) PHGPx activity and lower GPx activity [15]. Vitamin E ($\alpha$-tocopherol) concentration has been shown to increase with age in liver, adipose tissue and some brain areas in rats [16]. The influence of selenium on PHGPx activity has been investigated [17]. However, only a few studies have focused on variations with age in both PHGPx activity and membrane vitamin E level and still less on their simultaneous variations with both age and diet. Moreover, peroxidation of membrane lipids mainly depends on their fatty acid composition [18] and detailed studies dealing with age related variations of the latter are scarce [19].

Table 1
Fatty acid composition of dietary lipids

| Fatty acids (%, w/w) | Control (C) | Deficient (D) |
|---|---|---|
| 16:0 | 7.8 | 10.0 |
| 18:0 | 2.6 | 3.2 |
| 20:0 | 0.9 | 1.3 |
| 22:0 | 13 | 2.3 |
| 24:0 | 0.6 | 1.1 |
| 18:1n-9 | 58.1 | 58.3 |
| 18:1n-7 | 0.8 | 2.6 |
| 20:1n-9 | 1.2 | 1.2 |
| 18:2n-6 | 21.9 | 22.0 |
| 18:3n-3 | 4.2 | — |
| Saturated | 13.4 | 17.9 |
| Monounsaturated | 60.4 | 59.9 |
| n-6 polyunsaturated | 22.0 | 22.0 |
| n-3 polyunsaturated | 4.2 | — |
| n-6/n-3 ratio | 5.3 | 167.5 |

The lipid supply of each diet was 6.0 % by weight. The control diet contained a mixture (60:40) of peanut and rapeseed oils; the (n-3)-deficient diet contained peanut oil. Fatty acids contributing to less than 0.05% were omitted (—).

On the other hand, oxidized phospholipids very likely play an important role in atherosclerosis [20], numerous studies have shown a protective effect of PUFA against this disease and many of them suggested a possible major role of n-3 PUFA [21]. In addition, a modulating effect of PUFA on the age-related variations of glutathione metabolism and

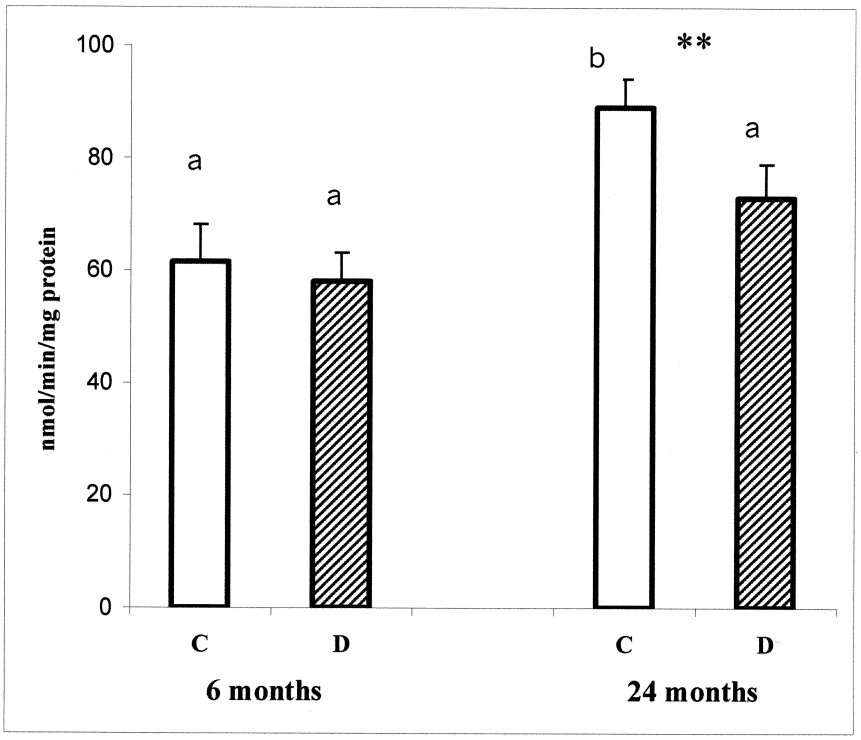



Fig. 1. PHGPx activity in rat liver microsomal membrane. Age (6 months vs. 24 months) and diet (control, C □ ; deficient, D ▨ ) induced variations. Values are means ± SEM, n = 8. Asterisks indicate an age effect (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars not bearing the same superscript letter are significantly different ($P < 0.05$).

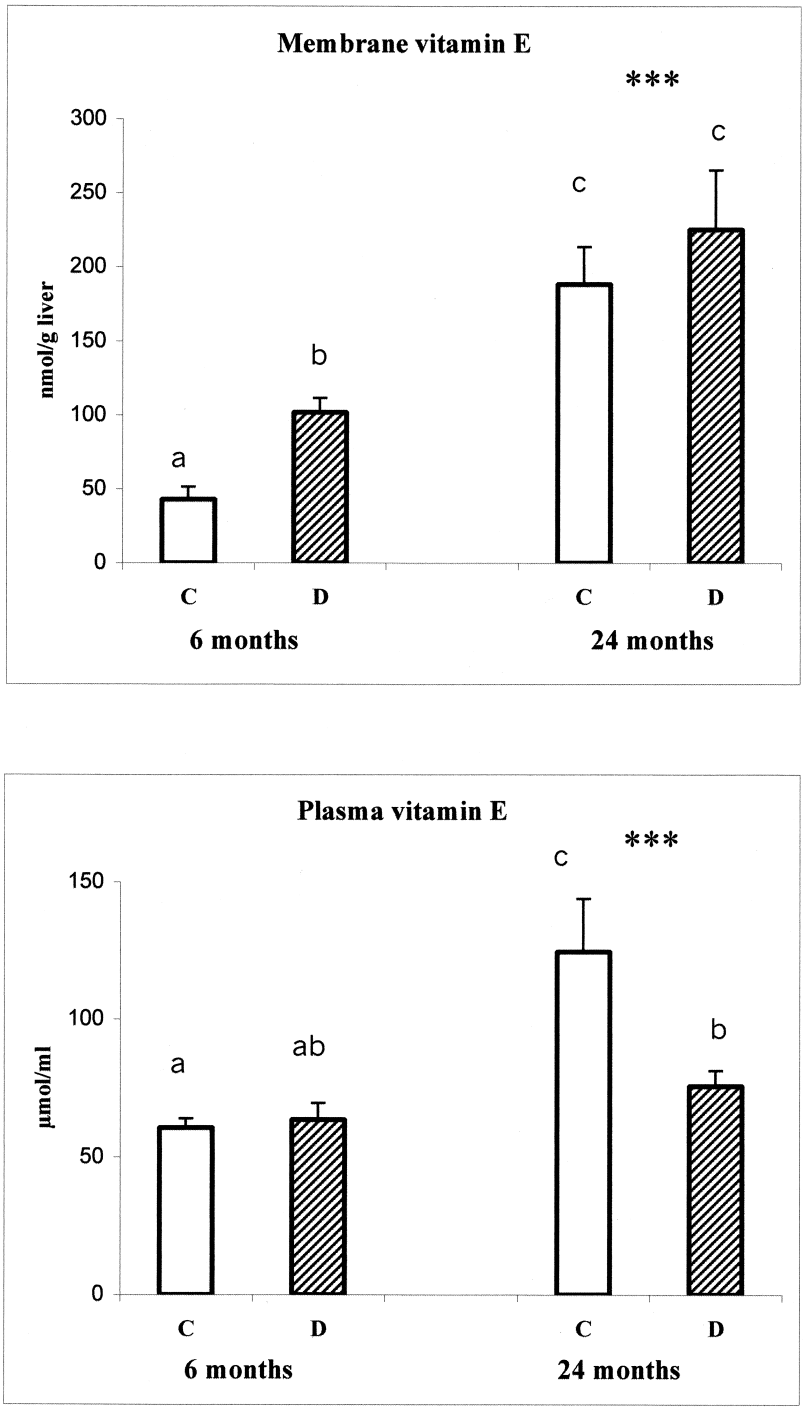



Fig. 2. Vitamin E concentration in rat plasma and liver microsomal membrane. Age (6 months vs. 24 months) and diet (control, C □ ; deficient, D ▨ ) induced variations. Values are means ± SEM, n = 8. Asterisks indicate an age effect (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars not bearing the same superscript letter are significantly different ($P < 0.05$).

GPx activity has previously been reported [22]. Therefore, the effect of n-3 PUFA deficiency has thus been investigated in young adult (6-month old) and old (24-month old) rats in order to study the possible diet-induced modulation of PHGPx activity and membrane vitamin E level, concomitantly with the effect of age.

## 2. Materials and methods

### *2.1. Nutritional procedures*

Male Wistar rats, inbred in our laboratory, were used. For two generations the animals were given a semipuri-

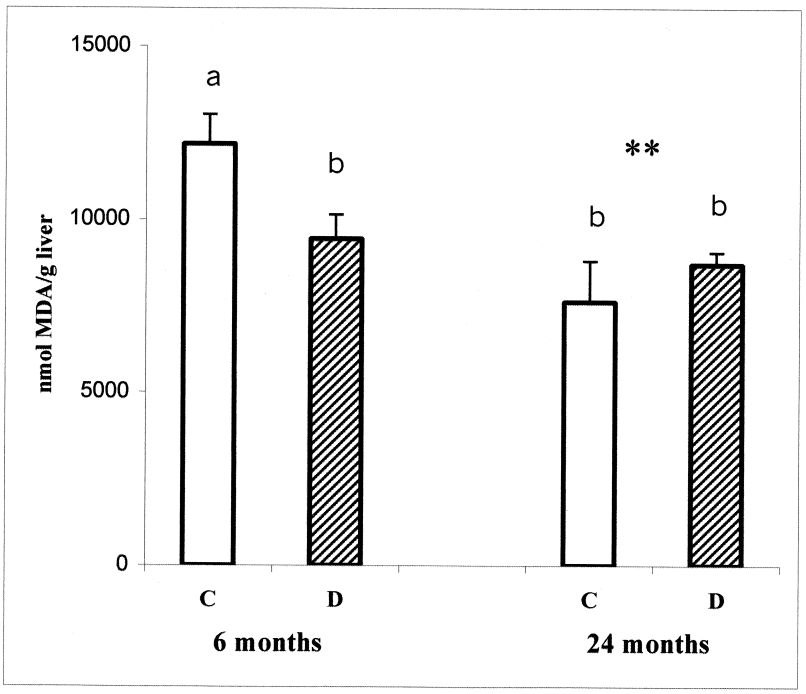



Fig. 3. Thiobarbituric acid reactive substances in rat liver microsomal membrane. Age (6 months vs. 24 months) and diet (control, C □ ; deficient, D ▨ ) induced variations. Values are means ± SEM, n = 8. Asterisks indicate an age effect (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars not bearing the same superscript letter are significantly different ($P < 0.05$).

fied standard diet containing 6% of either a mixture (60:40) of peanut and rapeseed oils (Control) or only peanut oil (Deficient). They were kept at 22 ± 1°C, with equal 12 h periods of light and dark and given free access to water and diet. According to the fatty acid composition of dietary lipids (Table 1), the control diet corresponded to a fatty acid balanced diet (supplying 1140 mg linoleic acid and 218 mg α-linolenic acid per 100 g of food) whereas the other diet was n-3 PUFA-deficient since it supplied 1104 mg linoleic acid and only 5 mg α-linolenic acid. Thus the latter diet led to a chronic α-linolenic acid deficiency. Eight male rats from each group were slaughtered at the age of 6 months, then 8 others at the age of 24 months, after 12 hr of fasting. All rats were sacrificed between 0800 h and 0900 h in order to take into account nycthemeral variations likely to affect certain parameters measured [23]. Blood was collected, immediately centrifuged and the plasma was stored at −80°C. The liver was rapidly removed, washed in ice-cold saline and frozen in liquid nitrogen prior to storage at −80°C.

### *2.2. Membrane preparation and chemical determinations*

A 10% (w/v) liver homogenate was prepared in ice-cold 3 mmol EDTA, 154 mmol KCI at pH 7.4 and the microsomes were isolated as previously reported [22]. The pellet was resuspended in ice-cold buffer (50 mmol Tris, 50 mmol maleate, 100 mmol KCL, pH 7.4) and then the membrane vesicles were frozen in aliquots in liquid nitrogen and stored at -80°C until analysis. Protein was estimated according to Bradford [24], using bovine serum albumin as a standard. Microsomal PHGPx activity was assayed by the procedure of Maiorino et al. [25] using phosphatidylcholine hydroperoxides (PC-OOH) as substrate. First, PC-OOH were prepared accordingly, using 0.04 mg/ml soybean lipoxigenase (Sigma, type IV), 0.3 mmol soybean phosphatidylcholine (Signia type III-S), 3 mmol sodium deoxycholate and 0.2 mol sodium borate (pH 9) in a 2.5 ml reaction mixture. Triton X-100 (Aldrich) was added to a final concentration of 0.2% (v/v) in order to solubilize microsomes and then the enzyme activity was measured by a coupled reaction using an excess of glutathione reductase and monitoring the NADPH oxidation at 340 nmol [26]. PHGPx activity was expressed as nmol NADPH oxidized/min/mg microsomal protein. The α-tocopherol concentration was determined in plasma and liver microsomal membrane by the method of Vuillemier et al. [27], using a high-performance liquid chromatography (HPLC) procedure. Determination was carried out at 280 nmol on lipid extracts, using α-tocopheryl acetate as an internal standard. Microsomal suspension was first saponified before extraction with hexane and separation of neutral lipid fraction. Thiobarbituric acid-reactive substances (TBARS) were measured on liver microsomal membranes, according to Parinandi et al. [28] and expressed as nmol MDA/g wet tissue.

### *2.3. Lipid analysis*

Lipid composition of liver microsomes was determined as previously described [29]. Total lipids were

Table 2
Fatty acid composition of phosphatidylcholine in the rat liver microsomal membrane as affected by age and dietary α-linolenic acid deficiency

| Fatty acids (%, w/w) | 6 months | | 24 months | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Control | Deficient | Control | Deficient | Age | Diet | A × D |
| 14:0 | 0.26 ± 0.03 | 0.26 ± 0.02 | 0.31 ± 0.01 | 0.35 ± 0.04 | | | |
| 15:0 | 0.18 ± 0.02 | 0.17 ± 0.02 | 0.19 ± 0.01 | 0.18 ± 0.02 | | | |
| 16:0 | 17.29 ± 0.63$^{a}$ | 14.86 ± 0.45$^{b}$ | 16.83 ± 0.58$^{a,c}$ | 15.36 ± 0.51$^{b,c}$ | | ** | |
| 17:0 | 0.30 ± 0.02 | 0.31 ± 0.01 | 0.33 ± 0.02 | 0.29 ± 0.01 | | | |
| 18:0 | 13.69 ± 0.63$^{a}$ | 13.71 ± 0.33$^{a}$ | 13.91 ± 0.61$^{a}$ | 16.58 ± 0.70$^{b}$ | * | * | * |
| 20:0 | 0.15 ± 0.03 | 0.13 ± 0.03 | 0.13 ± 0.02 | 0.16 ± 0.03 | | | |
| 22:0 | 0.14 ± 0.02 | 0.15 ± 0.02 | 0.17 ± 0.02 | 0.17 ± 0.02 | | | |
| 24:0 | 0.23 ± 0.02$^{a}$ | 0.21 ± 0.03$^{a}$ | 0.31 ± 0.03$^{b}$ | 0.26 ± 0.02$^{a,b}$ | ** | | |
| Σ SFA | 32.14 ± 0.97 | 29.73 ± 0.73 | 32.14 ± 0.91 | 33.30 ± 0.93 | | | |
| 16:1n-9 | 0.29 ± 0.01 | 0.34 ± 0.04 | 0.36 ± 0.02 | 0.34 ± 0.03 | | | |
| 16:1n-7 | 0.71 ± 0.04$^{a}$ | 0.57 ± 0.03$^{b}$ | 0.71 ± 0.01$^{a}$ | 0.58 ± 0.03$^{b}$ | | *** | |
| 18:1n-9 | 7.06 ± 0.14$^{a}$ | 7.61 ± 0.23$^{a,b}$ | 8.06 ± 0.21$^{b}$ | 8.48 ± 0.25$^{b}$ | *** | * | |
| 18:1n-7 | 3.75 ± 0.16$^{a}$ | 3.51 ± 0.20$^{a}$ | 3.46 ± 0.12$^{a}$ | 2.68 ± 0.14$^{b}$ | ** | ** | |
| 20:1n-9 | 0.26 ± 0.05 | 0.27 ± 0.03 | 0.27 ± 0.02 | 0.28 ± 0.04 | | | |
| 20:1n-7 | 0.27 ± 0.05 | 0.24 ± 0.02 | 0.30 ± 0.03 | 0.35 ± 0.05 | | | |
| 22:1n-9 | — | — | — | — | | | |
| 24:1n-9 | 0.20 ± 0.03$^{a}$ | 0.23 ± 0.03$^{a}$ | 0.38 ± 0.07$^{b}$ | 0.24 ± 0.03$^{a}$ | * | * | |
| Σ MUFA | 12.51 ± 0.25 | 12.79 ± 0.28 | 13.49 ± 0.29 | 12.93 ± 0.33 | | | |
| 20:3n-9 | 0.18 ± 0.03 | 0.13 ± 0.02 | 0.14 ± 0.02 | 0.20 ± 0.02 | | | |
| 18:2n-6 | 7.10 ± 0.37$^{a}$ | 6.90 ± 0.25$^{a}$ | 8.89 ± 0.38$^{b}$ | 8.20 ± 0.22$^{b}$ | *** | | |
| 20:2n-6 | 0.13 ± 0.03 | 0.10 ± 0.03 | 0.10 ± 0.00 | 0.13 ± 0.02 | | | |
| 20:3n-6 | 0.57 ± 0.06$^{a}$ | 0.23 ± 0.02$^{b}$ | 0.59 ± 0.05$^{a}$ | 0.35 ± 0.04$^{c}$ | | *** | |
| 20:4n-6 | 36.95 ± 1.01$^{a}$ | 43.09 ± 0.43$^{b}$ | 32.74$^{c}$ ± 0.61 | 38.14$^{a}$ ± 0.82 | *** | *** | |
| 22:4n-6 | 0.13 ± 0.02$^{a}$ | 0.41 ± 0.01$^{b}$ | 0.19 ± 0.01$^{a}$ | 0.48 ± 0.05$^{b}$ | | *** | |
| 22:5n-6 | 0.48 ± 0.11$^{a}$ | 3.73 ± 0.21$^{b}$ | 0.51 ± 0.06$^{a}$ | 330 ± 0.17$^{b}$ | | *** | |
| Σ n-6 PUFA | 47.50 ± 1.56$^{a}$ | 54.44 ± 0.67$^{b}$ | 42.99 ± 0.70$^{c}$ | 50.58 ± 0.80$^{a}$ | *** | *** | |
| 18:3n-3 | — | — | — | — | | | |
| 20:5n-3 | 0.27 ± 0.03$^{a}$ | 0.15 ± 0.03$^{b}$ | 0.53 ± 0.08$^{c}$ | 0.13 ± 0.02$^{b}$ | * | *** | * |
| 22:5n-3 | 0.47 ± 0.03$^{a}$ | 0.19 ± 0.03$^{b}$ | 0.79 ± 0.08$^{c}$ | 0.23 ± 0.02$^{b}$ | *** | *** | * |
| 22:6n-3 | 8.67 ± 0.38$^{a}$ | 2.59 ± 0.14$^{b}$ | 9.84 ± 0.17$^{c}$ | 2.65 ± 0.11$^{b}$ | ** | *** | * |
| Σ n-3 PUFA | 9.40 ± 0.35$^{a}$ | 2.89 ± 0.18$^{b}$ | 11.16 ± 0.25$^{c}$ | 2.99 ± 0.13$^{b}$ | *** | *** | ** |
| Σ PUFA | 55.13 ± 0.94$^{a,b}$ | 57.33 ± 0.64$^{b}$ | 54.14 ± 0.79$^{a}$ | 53.56 ± 0.90$^{a}$ | * | | |
| n-6/n-3 | 4.87 ± 0.20$^{a}$ | 19.41 ± 1.44$^{b}$ | 3.86 ± 0.10$^{a}$ | 17.10 ± 0.60$^{c}$ | ** | *** | |
| 22:5/22:6 | 0.06 ± 0.01$^{a}$ | 1.47 ± 0.11$^{b}$ | 0.05 ± 0.01$^{a}$ | 1.27 ± 0.09$^{b}$ | | *** | |
| DBI | 235.85 ± 5.10$^{a}$ | 237.34 ± 2.44$^{a}$ | 233.50 ± 2.83$^{a}$ | 219.74 ± 3.85$^{b}$ | * | | * |

Fatty acids are designated as in Table 1. Values (wt %) are means ± SEM for eight rats per dietary group. After variance analysis, the effects of age (A), diet (D) and age-diet interaction (A × D) are indicated as: *, $P < 0.05$ ; **, $P < 0.01$; ***, $P < 0.001$. Means were compared using the Scheffe F test and values in the same row sharing different superscript letters are significantly different. DBI: double bond index = Σ (mol% each unsaturated fatty acid × number of double bonds of the same fatty acid); SPA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.

extracted using chloroform/methanol (2:1, v/v) solvent containing 0.05% (w/v) butylated hydroxytoluene as antioxidant. Phospholipids were separated from neutral lipids on a silicic acid column. Cholesterol was assessed from the neutral lipid fraction using an enzymatic method (Boehringer Mannheim test kit). Phospholipid classes were separated by HPLC (Beckman Instruments, Gagny, France); their purity was checked using thin layer chromatography and quantitative estimation was performed by determination of inorganic phosphorus. Phospholipids were then transmethylated and the fatty acid methyl esters obtained were analyzed by gas chromatography using a Carlo Erba chromatograph (Rueil Malmaison, France) equipped with an automatic on-column injector, a flame-ionization detector and a Carbowax CP Wax 52 CB bonded fused-silica capillary column (50 m x 0.32 mm inner diameter). Data were processed using a Stang microcomputer (Pavillons-sous-Bois, France) and Nelson software (Cupertino, CA, USA).

### 2.4. Chemicals

All the chemicals used were of analytical reagent grade and purchased from Sigma-Aldrich Company.

### 2.5. Statistics

Statistical analyses were carried out using two-way analysis of variance (ANOVA) and Scheffe F test, $P < 0.05$ being considered significant. Results are reported as means ± SEM for eight animals.

Table 3
Fatty acid composition of phosphatidylethanolamine in the rat liver microsomal membrane as affected by age and dietary α-linolenic acid deficiency

| Fatty acids (%, w/w) | 6 months | | 24 months | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Control | Deficient | Control | Deficient | Age | Diet | A × D |
| 14:0 | 0.10 ± 0.00 | 0.28 ± 0.18 | 0.17 ± 0.02 | 0.13 ± 0.03 | | | |
| 15:0 | — | — | 0.10 ± 0.00 | — | | | |
| 16:0 | 12.96 ± 0.88$^{ab}$ | 12.01 ± 0.72$^{a}$ | 15.31 ± 1.36$^{b}$ | 11.36 ± 0.38$^{a}$ | | ** | |
| 17:0 | 0.27 ± 0.03 | 0.34 ± 0.03 | 0.33 ± 0.02 | 0.26 ± 0.02 | | | |
| 18:0 | 14.94 ± 0.56 | 15.63 ± 1.11 | 17.99 ± 135 | 15.59 ± 0.63 | | | |
| 20:0 | 0.13 ± 0.03 | 0.13 ± 0.03 | 0.20 ± 0.02 | 0.10 ± 0.00 | | | |
| 22:0 | — | — | — | — | | | |
| 24:0 | 0.16 ± 0.04 | — | 0.20 ± 0.02 | 0.12 ± 0.02 | | | |
| Σ SFA | 28.43 ± 0.93$^{a}$ | 28.25 ± 120$^{a}$ | 34.21 ± 2.12$^{b}$ | 27.43 ± 0.91$^{a}$ | | * | * |
| 16:1n-9 | 0.10 ± 0.00 | 0.12 ± 0.02 | 0.19 ± 0.03 | 0.11 ± 0.01 | | | |
| 16:1n-7 | 0.46 ± 0.05 | 0.43 ± 0.03 | 0.51 ± 0.04 | 0.43 ± 0.03 | | | |
| 18:1n-9 | 5.57 ± 0.49$^{a}$ | 8.71 ± 0.35$^{b}$ | 6.54 ± 0.51$^{a}$ | 8.78 ± 0.68$^{b}$ | | *** | |
| 18:1n-7 | 3.34 ± 0.22$^{a}$ | 3.35 ± 0.21$^{a}$ | 2.77 ± 0.14$^{b}$ | 2.58 ± 0.10$^{b}$ | | | |
| 20:1n-9 | 0.29 ± 0.03 | 0.35 ± 0.04 | 0.27 ± 0.04 | 0.26 ± 0.02 | | | |
| 20:1n-7 | 0.23 ± 0.03 | 0.23 ± 0.02 | 0.29 ± 0.03 | 0.33 ± 0.05 | | | |
| 22:1n-9 | — | — | — | — | | | |
| 24:1n-9 | 0.13 ± 0.03 | 0.15 ± 0.05 | 0.15 ± 0.05 | 0.10 ± 0.00 | | | |
| Σ MUFA | 10.04 ± 0.68$^{a}$ | 13.18 ± 0.37$^{b}$ | 10.61 ± 0.65$^{ab}$ | 12.50 ± 0.77$^{b}$ | | *** | |
| 20:3n-9 | 0.23 ± 0.02 | 0.25 ± 0.03 | 0.24 ± 0.04 | 0.45 ± 0.03 | | | |
| 18:2n-6 | 5.20 ± 0.45$^{a}$ | 6.04 ± 0.27$^{ab}$ | 6.56 ± 0.55$^{ab}$ | 6.83 ± 0.29$^{b}$ | * | | |
| 20:2n-6 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.11 ± 0.01 | 0.10 ± 0.00 | | | |
| 20:3n-6 | 0.50 ± 0.06 | 0.30 ± 0.02 | 0.47 ± 0.04 | 0.40 ± 0.04 | | | |
| 20:4n-6 | 34.47 ± 1.08$^{a}$ | 39.31 ± 0.60$^{b}$ | 27.70 ± 1.78$^{c}$ | 39.45 ± 0.97$^{b}$ | ** | *** | ** |
| 22:4n-6 | 0.51 ± 0.03$^{a}$ | 1.33 ± 0.08$^{b}$ | 0.56 ± 0.04$^{a}$ | 1.45 ± 0.04$^{b}$ | | *** | |
| 22:5n-6 | 0.74 ± 0.14$^{a}$ | 6.39 ± 0.63$^{b}$ | 0.77 ± 0.14$^{a}$ | 6.26 ± 0.41$^{b}$ | | *** | |
| Σ n-6 PUFA | 41.46 ± 1.11$^{a}$ | 53.43 ± 1.07$^{b}$ | 36.17 ± 1.72$^{c}$ | 54.41 ± 1.07$^{b}$ | | *** | * |
| 18:3n-3 | 0.10 ± 0.00 | — | 0.06 ± 0.03 | — | | | ** |
| 20:5n-3 | 0.36 ± 0.06$^{a}$ | 0.20 ± 0.04$^{a}$ | 0.89 ± 0.14$^{b}$ | 0.10 ± 0.00$^{c}$ | * | *** | |
| 22:5n-3 | 1.26 ± 0.13$^{a}$ | 0.33 ± 0.03$^{b}$ | 1.60 ± 0.14$^{a}$ | 0.45 ± 0.02$^{c}$ | * | *** | |
| 22:6n-3 | 18.16 ± 0.98$^{a}$ | 4.38 ± 0.27$^{b}$ | 16.16 ± 0.52$^{a}$ | 4.65 ± 0.21$^{b}$ | | *** | * |
| Σ n-3 PUFA | 19.77 ± 1.04$^{a}$ | 4.80 ± 0.32$^{b}$ | 18.64 ± 0.52$^{a}$ | 5.16 ± 0.23$^{b}$ | | *** | |
| Σ PUFA | 61.23 ± 0.97$^{a}$ | 58.23 ± 1.08$^{a}$ | 54.81 ± 1.98$^{b}$ | 59.58 ± 1.08$^{a}$ | | | ** |
| n-6/n-3 | 2.15 ± 0.17$^{a}$ | 11.46 ± 0.78$^{b}$ | 1.94 ± 0.09$^{a}$ | 10.70 ± 0.56$^{b}$ | | *** | |
| 22:5/22:6 | 0.04 ± 0.01$^{a}$ | 1.52 ± 0.21$^{b}$ | 0.05 ± 0.01$^{b}$ | 1.36 ± 0.09$^{b}$ | | *** | |
| DBI | 283.36 ± 5.25$^{a}$ | 249.89 ± 5.05$^{b}$ | 252.36 ± 8.82$^{a}$ | 254.13 ± 4.32$^{b}$ | * | * | ** |

See Table 2.

## 3. Results

### 3.1. PHGPx activity

In the liver microsomal membranes, the specific activity of PHGPx was significantly higher in 24-month old rats than in 6-month old rats (Figure 1). However, this effect of age was mainly due to the control diet, which accounted for a 45% increase ($P < 0.01$) as opposed to only 26% (non significant) for the deficient diet. PHGPx activity was positively correlated with vitamin E levels ($P < 0.05$) in the liver microsomal membrane ($r = 0.42$) and plasma ($r = 0.51$).

### 3.2. Vitamin E content

The vitamin E content of liver microsomal membrane (Figure 2) was markedly higher in the 24-month old rats than in the 6-month old rats ($P < 0.001$). In the latter, the vitamin E content was higher ($P < 0.001$) in the n-3 PUFA deficient rats than in the control rats. In the 24-month old rats, membrane vitamin E levels were not significantly different between the two dietary groups. Consequently, the increase during aging differed depending on the dietary lipids, i.e. it was considerably higher for the control diet (+340%) than for the deficient diet (+120%).

In the plasma, vitamin E levels *(Figure 2)* were similar in control and deficient 6-month old rats but higher in the older animals ($P < 0.001$), though more markedly in the control group. Therefore, a significant age-related increase was observed in the rats fed the control diet and not in those fed the deficient diet, which indicates a significant age-diet interaction.

The membrane to plasma ratio of tocopherol concentrations was increased (doubled) by both the α-linolenic acid deficiency and the age of rats. Furthermore, a positive correlation was observed between membrane and plasma levels of α-tocopherol ($P < 0.001$; $r = 0.57$).

Table 4
Fatty acid composition of phosphatidylinositol in the rat liver microsomal membrane as affected by age and dietary α-linolenic acid deficiency

| Fatty acids (%, w/w) | 6 months | | 24 months | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Control | Deficient | Control | Deficient | Age | Diet | A × D |
| 14:0 | 0.17 ± 0.02 | 0.21 ± 0.03 | 0.25 ± 0.03 | 0.20 ± 0.01 | | | |
| 15:0 | — | 0.13 ± 0.03 | — | — | | | |
| 16:0 | 6.13 ± 0.45$^{ab}$ | 4.94 ± 0.31$^{a}$ | 7.45 ± 0.34$^{c}$ | 6.18 ± 0.38$^{b}$ | ** | ** | |
| 17:0 | 0.34 ± 0.05 | 0.31 ± 0.03 | 0.43 ± 0.03 | 0.32 ± 0.02 | | | |
| 18:0 | 29.10 ± 1.14$^{a}$ | 26.61 ± 1.59$^{a}$ | 34.12 ± 0.67$^{b}$ | 25.76 ± 1.24$^{a}$ | | *** | * |
| 20:0 | 0.22 ± 0.07 | 0.20 ± 0.03 | 0.14 ± 0.02 | 0.17 ± 0.02 | | | |
| 22:0 | 0.10 ± 0.00 | 0.13 ± 0.03 | 0.10 ± 0.00 | 0.13 ± 0.03 | | | |
| 24:0 | 0.10 ± 0.00 | — | 0.10 ± 0.00 | — | | | |
| Σ SFA | 36.26 ± 1.69$^{a}$ | 32.39 ± 1.83$^{a}$ | 42.74 ± 1.06$^{b}$ | 32.46 ± 0.90$^{a}$ | * | *** | * |
| 16:1n-9 | 0.13 ± 0.02 | 0.21 ± 0.04 | 0.18 ± 0.04 | 0.17 ± 0.03 | | | |
| 16:1n-7 | 0.29 ± 0.03$^{ab}$ | 0.24 ± 0.02$^{a}$ | 0.40 ± 0.05$^{b}$ | 0.29 ± 0.02$^{a}$ | * | * | |
| 18-1n-9 | 4.20 ± 0.13$^{a}$ | 4.71 ± 0.23$^{a}$ | 6.06 ± 0.33$^{b}$ | 4.64 ± 0.16$^{a}$ | *** | | *** |
| 18:1n-7 | 1.03 ± 0.10 | 0.90 ± 0.10 | 0.91 ± 0.06 | 0.93 ± 0.11 | | | |
| 20:1n-9 | 0.17 ± 0.02 | 0.21 ± 0.03 | 0.19 ± 0.04 | 0.15 ± 0.02 | | | |
| 20:1n-7 | 0.23 ± 0.02$^{ab}$ | 0.15 ± 0.02$^{b}$ | 0.24 ± 0.03$^{a}$ | 0.19 ± 0.02$^{ab}$ | | * | |
| 22:1n-9 | — | — | — | — | | | |
| 24:1n-9 | 0.18 ± 0.05 | 0.15 ± 0.03 | 0.20 ± 0.00 | 0.13 ± 0.03 | | | |
| Σ MUFA | 5.91 ± 0.26$^{a}$ | 6.41 ± 0.27$^{a}$ | 7.83 ± 0.38$^{b}$ | 6.33 ± 0.11$^{a}$ | ** | | *** |
| 20:3n-9 | 1.30 ± 0.12 | 1.49 ± 0.12 | 1.24 ± 0.19 | 2.01 ± 0.24 | | | |
| 18:2n-6 | 4.11 ± 0.35$^{a}$ | 4.24 ± 0.26$^{a}$ | 6.45 ± 0.46$^{b}$ | 5.78 ± 0.28$^{b}$ | *** | | |
| 20:2n-6 | — | — | — | — | | | |
| 20:3n-6 | 2.01 ± 0.24$^{a}$ | 1.11 ± 0.08$^{b}$ | 1.83 ± 0.23$^{a}$ | 1.59 ± 0.19$^{ab}$ | | ** | |
| 20:4n-6 | 45.67 ± 1.01$^{ab}$ | 48.74 ± 1.34$^{b}$ | 33.27 ± 0.83$^{c}$ | 44.97 ± 0.49$^{a}$ | *** | *** | *** |
| 22:4n-6 | 0.39 ± 0.03$^{a}$ | 0.76 ± 0.05$^{b}$ | 0.41 ± 0.04$^{a}$ | 0.73 ± 0.04$^{b}$ | | *** | |
| 22:5n-6 | 0.50 ± 0.08$^{a}$ | 2.80 ± 0.24$^{b}$ | 0.54 ± 0.06$^{a}$ | 2.78 ± 0.17$^{b}$ | | *** | |
| Σ n-6 PUFA | 52.57 ± 1.15$^{a}$ | 57.66 ± 1.62$^{b}$ | 42.13 ± 1.27$^{c}$ | 54.84 ± 1.01$^{ab}$ | *** | *** | ** |
| 18:3n-3 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.14 ± 0.03 | 0.10 ± 0.00 | | | |
| 20:5n-3 | 0.17 ± 0.03 | — | 0.29 ± 0.04 | 0.18 ± 0.05 | | | |
| 22:5n-3 | 0.89 ± 0.09$^{a}$ | 0.23 ± 0.02$^{b}$ | 1.19 ± 0.08$^{c}$ | 0.28 ± 0.02$^{d}$ | ** | *** | * |
| 22:6n-3 | 6.66 ± 0.51$^{a}$ | 1.60 ± 0.14$^{b}$ | 6.59 ± 0.46$^{a}$ | 1.59 ± 0.12$^{b}$ | | *** | |
| Σ n-3 PUFA | 7.54 ± 0.59$^{a}$ | 1.83 ± 0.15$^{b}$ | 7.81 ± 0.46$^{a}$ | 1.86 ± 0.13$^{b}$ | | *** | |
| Σ PUFA | 60.15 ± 1.67$^{a}$ | 59.49 ± 1.68$^{a}$ | 48.65 ± 2.31$^{b}$ | 56.23 ± 1.07$^{a}$ | *** | | * |
| n-6/n-3 | 7.18 ± 0.56$^{a}$ | 32.80 ± 2.80$^{b}$ | 5.49 ± 0.19$^{c}$ | 28.66 ± 1.80$^{b}$ | | *** | |
| 22:5/22:6 | 0.08 ± 0.01$^{a}$ | 1.87 ± 0.31$^{b}$ | 0.08 ± 0.02$^{a}$ | 1.81 ± 0.15$^{b}$ | | *** | |
| DBI | 260.28 ± 8.56$^{a}$ | 245.67 ± 6.84$^{ac}$ | 210.20 ± 3.55$^{b}$ | 235.70 ± 3.65$^{c}$ | *** | | ** |

See Table 2.

### 3.3. Membrane peroxidability

Following in vitro stimulation of membrane peroxidation, the production of TBARS significantly decreased with age (Figure 3). However, in the 6-month old rats, the MDA content was lower for the deficient diet ($P < 0.05$) than for the control diet, whereas no variation was observed between dietary groups in the old rats. This led to a significant interaction ($P < 0.05$) between the age and diet effects. Thus, the decrease with age (which reached 37%) was closely linked to the control diet.

### 3.4. Lipid composition of microsomal membrane

No change appeared in total lipids (average 0.6 ± 0.1 mg/mg protein), total phospholipids (PL) (average 580 ± 85 nmol/mg protein) or total cholesterol (average 43 ± 3 nmol/mg protein). PL class distribution was also not significantly affected by either the diet or the rat age. The percentages of each main PL class were 51.2 ± 1.0 and 49.9 ± 1.0 for phosphatidylcholine (PC), 32.6 ± 0.8 and 34.7 ± 0.7 for phosphatidylethanolamine (PE), 14.1 ± 0.4 and 12.8 ± 0.5 for phosphatidylinositol (PI) and 2.2 ± 0.1 and 2.6 ± 0.1 for phosphatidylserine (PS), in 6-month old and 24-month old rats, respectively. However, the fatty acid composition of membrane PL was markedly modified by both age and dietary n-3 PUFA deficiency *(Tables 2 to 5)*.

Whatever the rat age, the α-linolenic acid deficiency led, in all main PL classes, to lower n-3 PUFA levels ($P < 0.001$), namely that of eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and, concomitantly, to higher n-6 PUFA levels ($P < 0.001$), mainly that of arachidonic (20:4n-6), docosatetraenoic (22:4n-6) and docosapentaenoic (22:5n-6) acids. Therefore, in all PL, n-6/n-3 and 22:5n-6/22:6n-3 ratios were higher ($P < 0.001$) in deficient diet rats than in control rats of both

Table 5
Fatty acid composition of phosphatidylserine in the rat liver microsomal membrane as affected by age and dietary α-linolenic acid deficiency

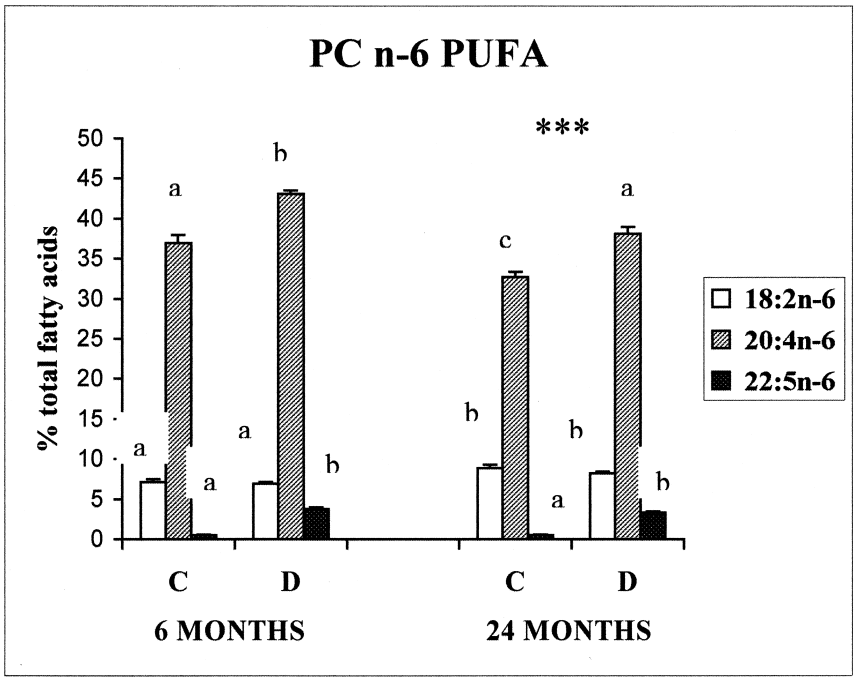
| Fatty acids (%, w/w) | 6 months | | 24 months | | Significance | | |
|---|---|---|---|---|---|---|---|
| | Control | Deficient | Control | Deficient | Age | Diet | A × D |
| 14:0 | 0.74 ± 0.12 | 0.75 ± 0.08 | 0.70 ± 0.08 | 0.72 ± 0.14 | | | |
| 15:0 | 0.29 ± 0.07 | 0.23 ± 0.02 | 0.24 ± 0.02 | 0.26 ± 0.04 | | | |
| 16:0 | 12.11 ± 1.09 | 10.35 ± 0.68 | 10.42 ± 1.04 | 8.95 ± 0.94 | | | |
| 17:0 | 0.38 ± 0.06 | 0.47 ± 0.04 | 0.42 ± 0.04 | 0.34 ± 0.04 | | | |
| 18:0 | 25.56 ± 1.28 | 29.50 ± 1.31 | 29.54 ± 1.10 | 30.48 ± 1.19 | | | |
| 20:0 | 0.73 ± 0.11 | 0.63 ± 0.11 | 0.52 ± 0.12 | 0.68 ± 0.07 | | | |
| 22:0 | 0.74 ± 0.13 | 0.67 ± 0.09 | 0.48 ± 0.04 | 0.63 ± 0.08 | | | |
| 24:0 | 0.89 ± 0.12 | 1.00 ± 0.15 | 0.90 ± 0.13 | 0.92 ± 0.11 | | | |
| Σ SFA | 41.64 ± 0.49 | 43.60 ± 0.98 | 43.22 ± 0.41 | 43.34 ± 0.68 | | | |
| 16:1n-9 | 0.88 ± 0.16 | 0.60 ± 0.10 | 0.70 ± 0.14 | 0.69 ± 0.09 | | | |
| 16:1n-7 | 0.76 ± 0.15 | 0.58 ± 0.09 | 0.52 ± 0.07 | 0.51 ± 0.06 | | | |
| 18:1n-9 | 11.46 ± 1.07 | 9.33 ± 0.46 | 9.36 ± 0.88 | 8.10 ± 0.91 | | | |
| 18:1n-7 | 1.44 ± 0.14 | 1.35 ± 0.17 | 1.28 ± 0.09 | 1.04 ± 0.08 | | | |
| 20:1n-9 | 0.90 ± 0.15 | 0.78 ± 0.22 | 0.50 ± 0.12 | 0.64 ± 0.06 | | | |
| 20:1n-7 | 0.56 ± 0.16 | 0.47 ± 0.16 | 0.52 ± 0.20 | 0.70 ± 0.10 | | | |
| 22:1n-9 | 0.21 ± 0.04 | 0.20 ± 0.04 | 0.16 ± 0.02 | 0.19 ± 0.03 | | | |
| 24:1n-9 | 0.48 ± 0.12 | 0.55 ± 0.11 | 0.64 ± 0.05 | 0.50 ± 0.07 | | | |
| Σ MUFA | 15.98 ± 1.07 | 13.87 ± 0.78 | 13.68 ± 0.94 | 12.30 ± 1.15 | | | |
| 20:3n-9 | 0.34 ± 0.05 | 0.28 ± 0.04 | 0.22 ± 0.04 | 0.38 ± 0.03 | | | |
| 18:2n-6 | 3.00 ± 0.28 | 2.55 ± 0.15 | 3.02 ± 0.39 | 3.44 ± 0.24 | | | |
| 20:2n-6 | 0.21 ± 0.04 | 0.23 ± 0.06 | 0.16 ± 0.02 | 0.22 ± 0.03 | | | |
| 20:3n-6 | 0.41 ± 0.06 | 0.33 ± 0.03 | 0.44 ± 0.02 | 0.38 ± 0.05 | | | |
| 20:4n-6 | 22.17 ± 1.85[a] | 27.25 ± 1.07[b] | 23.16 ± 1.29[a] | 28.28 ± 1.68[b] | | ** | |
| 22:4n-6 | 0.54 ± 0.03[a] | 0.87 ± 0.11[b] | 0.62 ± 0.06[a] | 1.11 ± 0.12[b] | | *** | |
| 22:5n-6 | 0.91 ± 0.07[a] | 4.07 ± 1.21[b] | 1.16 ± 0.11[a] | 5.28 ± 0.58[b] | | *** | |
| Σ n-6 PUFA | 27.40 ± 1.71[a] | 35.22 ± 1.50[b] | 28.56 ± 1.03[a] | 37.54 ± 1.00[b] | | *** | |
| 18:3n-3 | 0.41 ± 0.04 | 0.37 ± 0.02 | 0.38 ± 0.06 | 0.36 ± 0.04 | | | |
| 20:5n-3 | 0.30 ± 0.05[a] | 0.32 ± 0.10[a] | 0.54 ± 0.13[b] | 0.20 ± 0.05[a] | | * | ** |
| 22:5n-3 | 0.66 ± 0.05[a] | 0.38 ± 0.10[a] | 0.92 ± 0.09[c] | 0.40 ± 0.07[b] | | *** | |
| 22:6n-3 | 9.19 ± 0.39[a] | 3.53 ± 0.51[b] | 11.76 ± 0.66[c] | 3.00 ± 0.20[b] | * | *** | ** |
| Σ n-3 PUFA | 10.56 ± 0.46[a] | 4.25 ± 0.61[b] | 13.60 ± 0.58[c] | 3.83 ± 0.18[b] | * | *** | ** |
| Σ PUFA | 38.58 ± 1.55 | 38.05 ± 1.97 | 42.16 ± 1.21 | 40.03 ± 0.96 | | | |
| n-6/n-3 | 2.47 ± 0.20[a] | 9.34 ± 1.72[b] | 2.11 ± 0.11[a] | 9.19 ± 0.11[b] | | *** | |
| 22:5/22:6 | 0.10 ± 0.01[ab] | 1.82 ± 0.62[b] | 0.10 ± 0.01[a] | 1.89 ± 0.15[b] | | *** | |
| DBI | 186.37 ± 5.34[a] | 184.10 ± 4.11[a] | 201.94 ± 5.28[b] | 182.70 ± 4.38[a] | | * | |

See Table 2.

age groups. By contrast, SFA and MUFA levels were only slightly modified by the diet. Moreover, the fatty acid composition of phospholipids was also significantly affected by aging. This effect was most marked in PC, then in PI and also in PE, though to a lesser extent, and very slight in PS. PUFA were mainly concerned: in the first three PL classes of 24-month old rats, as compared to 6-month old rats, total n-6 PUFA decreased ($P < 0.001$ in PC and PI, $P < 0.10$ in PE), due to an important decrease in 20:4 level ($P < 0.01$ to 0.001), despite an increased linoleic acid level (18:2 n-6, $P < 0.001$ in PC and PI, $P < 0.05$ in PE). In PC (Figure 4), these changes in n-6 fatty acid profiles were observed whatever the diet whereas in PI and PS, they were only significant for the control diet. Concomitantly, n-3 PUFA levels were higher in the 24-month old rats than in the 6-month old rats, mainly in PC ($P < 0.001$) and PS ($P < 0.05$) and only in the rats fed the control diet. Consequently, compared with the adult, the old rats showed a lower n-6/n3 ratio (significantly in PC) and total PUFA level (significantly in PC and PI), and therefore a lower double bond index (DBI) in PC, PE and PI. Moreover, SFA and MUFA were also modified with age, but to a much lesser extent than PUFA. The changes essentially occurred in PC and PI (they were also observed in PE, but not at all in PS) and consisted of higher levels of total SFA and oleic acid (18:ln-9) in 24-month old rats as compared to the 6-month old rats, mainly those receiving the control diet. By contrast, the level of vaccenic acid (18:1n-7) in PC and PE was significantly lower in 24-month old rats than in 6-month old rats.

## 4. Discussion

The present data show for the first time that, though it was unchanged with the diet in the adult animals, the increase with age of PHGPx specific activity depended on the nature of dietary fatty acids. Our results clearly indicate that



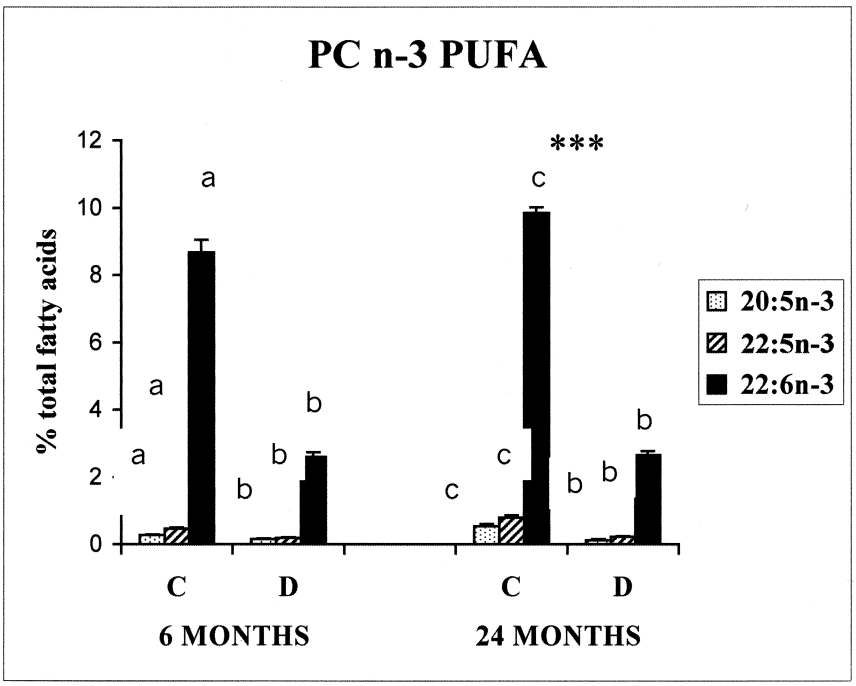




Fig. 4. Effect of age (6 months vs. 24 months) and $\alpha$-linolenic acid deficiency (control, C; deficient, D) on n-6 PUFA ( □ 18:2, ▨ 20:4 and ▩ 22:5) levels and n-3 PUFA (▨ 20:5, ▨ 22:5 and ■ 22:6) levels in PC of rat liver microsomal membrane. Values are means ± SEM, n = 8. Asterisks indicate an age effect (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$). Bars not bearing the same superscript letter are significantly different ($P < 0.05$).

the enzyme specific activity in liver microsomal membrane significantly increased with age, confirming those of Zhang et al. [30] who also found a 30% increase in the liver PHGPx specific activity of 24-month old rats as compared to 4-month old rats. D'Aquino et al. [14] had not shown any significant difference between the effects of coconut and fish oil based diets on PHGPx activity in young adult rats, but they did not study the concomitant effect of aging.

The $\alpha$-tocopherol concentration in rat liver and adipose tissue has been shown to increase with age [15]. In a longitudinal study involving 200 women and 231 men aged 20–95 years, Hallfrisch et al. [31] observed a higher plasma

α-tocopherol level in older people. Our data on rat liver and plasma vitamin E concentrations are consistent with these results as regards the age variations. They also clearly showed that α-tocopherol status was affected by both age and dietary fat type *(Figure 2)*. Plasma and liver α-tocopherol concentrations were lower in animals fed a fish oil diet than in those fed a corn or a coconut oil based diet [32]. This could result from a decreased absorption of vitamin E in fish oil-fed animals [10,33]. However, little is known about the relationship between dietary fatty acids and vitamin E absorption depending on age. In the 6-month old rats, our data show no effect of the dietary α-linolenic acid deficiency on the plasma vitamin E level but a significantly lower membrane level in the control rat liver than in the deficient rat liver. This supports most studies dealing with the greater requirement of membrane vitamin E as the unsaturation of dietary fatty acids is higher [9,18]. By contrast, in the 24-month old rats, the plasma vitamin E level was lower ($P < 0.05$) in the deficient rats than in the control rats, whereas no significant difference was observed in the membrane concentrations. Therefore, the plasma vitamin E level increased with age only in the rats fed the control diet while the membrane vitamin E concentration increased with age whatever the dietary treatments. The lower plasma vitamin E level in 24-month old deficient rats is unlikely related to the above mentioned depressive effect of unsaturation on vitamin E absorption. However, it can be observed that, in both the age groups of rats, the dietary n-3 PUFA deficiency led to approximately the same increase in the membrane to plasma ratio of α-tocopherol concentrations. An age-related increase in this ratio was also observed, whatever the diet. It may be assumed that, whatever the nature of dietary fatty acids, a high level of vitamin E is maintained in cellular membranes of aging animals. However, the mechanisms involved in this process have not yet been elucidated. Moreover, the positive correlation observed between PHGPx activity and liver vitamin E levels might confirm the synergic interaction reported previously between these two antioxidants [7,11].

The latter observations could be linked to the decrease in TBARS (measured after stimulated peroxidation) with aging which we showed here, confirming previous works [34,35]. Furthermore, this decrease is consistent with the decreased glutathione peroxidase activities recently reported in old rat liver as compared to adult rat liver [22]. However, in the present study, the age-related decrease in membrane peroxidability depended on the dietary fatty acids. According to some other studies [14], in the young-adult rats, MDA production was lower ($P < 0.05$) with the deficient diet than with the control diet, which may be directly related to dietary fatty acid unsaturation [18,29]. However, this was not observed in the 24-month old rats. Therefore, the age-related decrease in TBARS was only significant in the rats fed the control diet.

In fact, the membrane lipid composition was also altered by the nature of dietary PUFA and rat age. According to previous studies [19], the proportions of major PL classes were not significantly affected by age or diet, in contrast to their fatty acid composition. At both experimental ages, the α-linolenic acid deficiency led to a dramatic change in fatty acid profiles of PL. n-6 PUFA were increased (whereas n-3 PUFA were decreased) as is usual in the deficient rat microsomal membranes. This was more marked in the old rats than in the adults, likely due to the increasing paucity of n-3 fatty acid pools in the former. Therefore, the age-related modifications observed in the fatty acid composition of liver microsomal membranes were quite different depending on the dietary lipids. While the two dietary groups of 24-month old rats had significantly lower levels of n-6 PUFA (mainly arachidonic acid) than the 6-month old rats, higher n-3 PUFA (DHA, docosapentaenoic acid and EPA) levels were observed in the old rats than in the adult rats, only for the control diet. An age-related alteration in the fatty acid composition of microsomal membrane phospholipids has been reported in Fischer-344 rat thymocytes, without any studied effect of dietary fatty acids [36]. The lower 20:4n-6 level and the concomitant higher n-3 PUFA, 18:2n-6 and 18:1n-9 levels that we show in the old rats as compared to the adult rats are consistent with this work. Furthermore, these modifications are consistent with the age-related variations in desaturase activities previously reported [37], as 20:4n-6/18:2n-6 and 20:4n-6/20:3n-6 ratios (not shown) were clearly lower in the 24-month old rats than in the 6-month old rats. Δ6 desaturase specific activities for either linoleic or α-linolenic acids were thought to decline with age and the former seemed to be more affected than the latter [37]. Our results partly agree with this assumption which could explain the marked age-related decrease in 20:4n-6 level in most PL classes of rat liver microsomal membranes.

In conclusion, the significant increase in PHGPx specific activity and vitamin E content observed in rat liver microsomal membrane with aging is most likely related to a decrease in membrane peroxidability. This result may be linked to the age-related decrease in n-6 PUFA exhibited in the main membrane PL classes, despite a concomitant increase in n-3 PUFA levels. It is noteworthy that these results were mainly observed in the animals fed a diet balanced in linoleic and α-linolenic acids. Our results strongly suggest that a chronic n-3 PUFA deficiency limits the capacity of animals to compensate for the in vivo peroxidation naturally increasing with age.

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