# Inverse modifications of heart and liver α-tocopherol status by various dietary n-6/n-3 polyunsaturated fatty acid ratios[1]

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**Abstract** The effect of dietary n-6/n-3 fatty acid ratio on α-tocopherol homeostasis was investigated in rats. Animals were fed diets containing fat (17% w/w) in which the n-6/n-3 ratio varied from 50 to 0.8. This was achieved by combining corn oil, fish oil, and lard. The polyunsaturated to saturated ratio and total α-tocopherol remained constant in all diets. Results showed that enrichment of n-3 polyunsaturated fatty acids in the diet, even at a low amount (3.9% w/w), resulted in a dramatic reduction of blood α-tocopherol concentration, which, in fact, is the result of a decrease in plasma lipids, since the α-tocopherol to total lipids ratio was not significantly altered. The most striking effect observed was a considerable α-tocopherol enrichment (× 4) of the heart as its membranes became enriched with n-3 polyunsaturated fatty acids. This process appeared even with a low amount of fish oil (3.9% w/w) added to the diet. Accordingly, a strong positive correlation was found between heart α-tocopherol and docosahexaenoic acid ($r$ = 0.86) or docosahexaenoic acid plus eicosapentaenoic acid levels ($r$ = 0.84). Conversely, the liver α-tocopherol level dropped dramatically when n-3 polyunsaturated fatty acids were gradually added to the diet. It is concluded that fish oil intake dramatically alters the α-tocopherol homeostasis in rats. —**Chautan, M., R. Calaf, J. Léonardi, M. Charbonnier, M. Andre, H. Portugal, A-M. Pauli, H. Lafont, and G. Nalbone.** Inverse modifications of heart and liver α-tocopherol status by various dietary n-6/n-3 polyunsaturated fatty acid ratios. *J. Lipid Res.* 1990. **31:** 2201-2208.

**Supplementary key words** α-tocopherol • homeostasis

Because of their hypolipidemic properties (1, 2), consumption of polyunsaturated fatty acids (PUFA) of vegetable or marine origin has been recommended to lower the incidence of coronary diseases. Fish oils contain the longest chain polyunsaturated series, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), found in living systems. This makes them susceptible to peroxidation in vitro, as well as in vivo, once they are absorbed. Several studies have shown that DHA is efficiently incorporated into the major classes of membrane phospholipids of many organs including heart (3-8) which is due, in part, to the peculiar kinetic properties of heart acyl-CoA synthetase toward this fatty acid (9). As a consequence, heart membranes become highly enriched in DHA, which renders them more susceptible to the attack by free radicals such as the hydroxyl radical. Fortunately, cells have developed a multi-level system against lipid peroxidation in which vitamin E is thought to be one of the most important components because of its pivotal role in blocking the propagation of lipid peroxidation in the bilayer. Several animal studies have shown that increasing the level of polyunsaturated fatty acids supplied by dietary marine or vegetable oils has led to a higher peroxidative process in several organs such as liver (10, 11), heart (7, 8, 12), and kidney (10). This was shown by an increased storage of fluorescent pigments or lipofuscin (3, 7, 8, 12), thiobarbituric acid-reactive substances (10), or by susceptibility to in vitro-induced peroxidation (13). This pathological process can be prevented by increasing the dietary supply of vitamin E (11, 12) which argues for a higher demand of this vitamin under fish oil administration (14). In heart, membrane peroxidative injury is deleterious to its function. This is illustrated by the harmful effect of free radicals generated during reperfusion of the ischemic heart (for a review see ref. 15) and it has recently been shown that pretreatment of animals with vitamin E can afford some protection to the ischemic (16)

Abbreviations: PUFA, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA eicosapentaenoic acid; P/S ratio, polyunsaturated to saturated ratio; TG, triacylglycerol; ASAT, aspartate aminotransferase; ALAT, alanine aminotransferase; GSH-PX, selenium-dependent glutathione peroxidase.

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or hypertensive (17) myocardium. However, no study so far has demonstrated any detrimental effect of fish oil on cardiac function (18, 19), although in some other studies, fish oil was demonstrated to induce lipid peroxide deposition (7, 8, 12). Except for the problem of different experimental conditions (dose, animal, duration, etc.), this would suggest also that either the repair process of peroxidized phospholipids is very efficient or that lipoperoxides do not originate from membrane peroxidation but from another process.

Thus, the purpose of this study was to gain insight into the mechanisms by which the heart reacts against a diet-induced higher susceptibility to membrane peroxidation. To this end, we conducted dietary experiments in which the main variable was the n-6/n-3 PUFA ratio. The polyunsaturated to saturated (P/S) ratio was held constant as well as the α-tocopherol supply. Results showed that the heart α-tocopherol content dramatically increased as membranes become enriched in n-3 PUFA, whereas liver became depleted in α-tocopherol.

## MATERIALS AND METHODS

### Animals and feeding procedures

Four groups of seven male Wistar rats (IFFA Credo, L'Arbresle, France) weighing 300 ± 20 g were housed in pairs. Each group was fed a diet containing 17% (w/w) of fat (**Table 1**). A constant P/S ratio of about 2 was maintained in each diet, but not the n-6/n-3 ratio which varied from 50 to 0.8 (see **Table 2** for the fatty acid composition of diets). Also, diets were corrected for the same amount of cholesterol. As the overall amount of inherent α-tocopherol in the diets was above that usually used, the slight differences in each diet were not considered important. Diet A was prepared to last 1 month and stored at −20°C in plastic bags, sealed, and flushed under nitrogen. Other diets were prepared to last 2 weeks and stored under the same conditions as above. The rats were fed for 1 month ad libitum and the amount of uneaten food that was discarded in the morning did not show, over a 1-week period, any significant variation among the four groups of rats. Rats had free access to tap water.

TABLE 1. Composition of diets (g/100 g)

| | Diet A | Diet B | Diet C | Diet D |
|---|---|---|---|---|
| Casein[a] | 27.3 | 27.3 | 27.3 | 27.3 |
| Starch[a] | 26.9 | 26.9 | 26.9 | 26.9 |
| Glucose[a] | 18.0 | 18.0 | 18.0 | 18.0 |
| Minerals[a] | 5.0 | 5.0 | 5.0 | 5.0 |
| Vitamins[a] | 1.0 | 1.0 | 1.0 | 1.0 |
| Cellulose[a] | 4.8 | 4.8 | 4.8 | 4.8 |
| Corn oil[b] | 11.42 | 9.35 | 7.36 | 4.5 |
| Lard[a] | 5.58 | 3.74 | 2.16 | |
| Salmon oil[c] | | 3.91 | 7.48 | 12.5 |
| α-Tocopherol | 0.0192 | 0.0195 | 0.0199 | 0.0203 |
| γ-Tocopherol | 0.0099 | 0.0081 | 0.0064 | 0.0039 |
| Other tocopherols | 0.0006 | 0.0005 | 0.0004 | 0.0002 |
| α-Tocopheryl acetate | | 0.0008 | 0.0015 | 0.0025 |

α-Tocopherol content is the sum of what was in the vitamin mix plus that naturally contained in fat. α-Tocopherol was in corn oil, whereas α-tocopheryl acetate was in salmon oil. Because salmon oil and lard contained cholesterol, the amount was kept constant (0.08%) with added exogenous cholesterol.

[a]From Unité Alimentation Rationnelle, Villemoisson, France.
[b]From Grandes Huileries Metropolitaines, Marseille, France.
[c]From Unit Specific Aliment, St. Waast la Vallée, France.

TABLE 2. Fatty acid composition (weight %) of diets

| | Diet A | Diet B | Diet C | Diet D |
|---|---|---|---|---|
| 14:0 | 0.43 | 1.68 | 2.85 | 4.48 |
| 15:0 | | | 0.17 | 0.29 |
| 16:0 | 15.8 | 14.5 | 13.5 | 12.0 |
| 16:1 n-7 | 0.95 | 2.32 | 3.69 | 5.39 |
| 17:0 | | 0.20 | 0.35 | 0.59 |
| 18:0 | 5:27 | 4.15 | 3.37 | 2.34 |
| 18:1 n-9 | 34.8 | 28.4 | 22.7 | 14.8 |
| 18:1 n-7 | | 0.83 | 1.58 | 2.64 |
| 18:2 n-6 | 40.8 | 33.6 | | 16.3 |
| 18:3 n-6 | | | 0.15 | 26.6 |
| 18:3 n-3 | 0.80 | 0.63 | 0.65 | 0.68 |
| 18:4 n-3 | | 0.60 | 1.14 | 1.91 |
| 20:1 | 0.48 | 3.44 | 6.21 | 10.1 |
| 20:4 n-6 | | 0.14 | 0.26 | 0.44 |
| 20:5 n-3 | | 3.12 | 5.95 | 9.94 |
| 22:1 n-11 | | 2.10 | 4.00 | 6.68 |
| 22:1 n-9 | 0.39 | 0.44 | 0.5 | 0.59 |
| 22:5 n-3 | | 0.39 | 0.75 | 1.27 |
| 22:6 n-3 | | 2.25 | 4.31 | 7.26 |
| 24:1 n-9 | | 0.11 | 0.22 | 0.37 |
| n-6/n-3 | 51 | 4.63 | 2.03 | 0.78 |
| P/S | 1.92 | 1.95 | 1.99 | 1.98 |

### Tissue and blood treatments

At the end of the feeding period, the overnight-fasted rats were weighed and guillotined in the morning at the same time for each group. Blood was collected in heparin-containing tubes and centrifuged at 2,000 *g* for 20 min at 4°C. A portion of the plasma was immediately used for plasma parameter assays while the other part was aliquoted and stored at −30°C pending analysis for glutathione peroxidase and vitamin A and E assays. Heart and liver were quickly excised and submitted to lipid extraction as previously described (7). Butylated hydroxytoluene at 0.05% was added to the samples that were flushed with nitrogen and stored at −30°C.

### Lipid analysis, plasma parameters, and enzyme assays

The plasma lipids assayed were triglycerides (TG) (20), and total cholesterol (21). Plasma alanine aminotransferase (ALAT) (E.C. 2.6.1.2.) and aspartate aminotransferase (ASAT) (E.C. 2.6.1.1.) were assayed according to the method of Kessler et al. (22) and alkaline phosphatase (E.C. 3.1.3.1.) by the method of Morgenstern et al. (23). Plasma proteins (24) and albumin (25) were auto-

matically assayed. The method of Levander et al. (26) was used to assay the selenium-dependent glutathione peroxidase (GSH-PX) (E.C. 1.11.19.). Plasma α-, γ-tocopherol, and vitamin A were separated and assayed by high performance liquid chromatography on a RP8 LiChrosorb (7μ, 250 × 4 mm) column (Merck, Darmstadt, FRG) as described by De Leenher et al. (27). Detection was at 295 nm. Fatty acid methyl esters of total lipids isolated from heart and liver were prepared (28) and separated as described before (7). Lipid phosphorus was assayed as already published (29).

### Chemicals and statistical analysis

Fatty acid methyl ester standards (99% pure) were from Interchim (Paris, France) and standards of α-, γ-tocopherol, and vitamin A were from Sigma (La Verpillière, France). Statistical significance of mean differences between dietary groups was investigated by analysis of variance (ANOVA) and by the Sheffé multiple comparison method.

## RESULTS

### Plasma parameters (Table 3)

Over the 4-week feeding period no significant difference ($P > 0.05$) was observed in body weight gains: 192 ± 12.1 g; 207 ± 18.5 g; 222 ± 15.0 g; and 193 ± 21 g for rats belonging to groups A, B, C, and D, respectively. Total plasma protein and albumin concentrations were not significantly modified by diets, indicating that the overall nutritional status of the animals was not modified. Plasma transaminase activities did not show any marked variation among the four groups. Plasma alkaline phosphatase was the highest in rats fed diet D, this slight difference being significant only when compared with diet C (+36%). As these values were in the range of those found in rats fed low fat diets (7, 8), it can be concluded that various diets did not lead to significant cellular injury. Enrichment of diets with n-3 PUFA resulted in a marked drop in plasma lipids. Adding 3.9% fish oil to the diet (diet B) resulted in a significant decrease in plasma TG concentration (−34%) and the most important difference (−54%) was between diets A and D. A comparable effect of the n-6/n-3 PUFA ratio was observed with total cholesterol since similar percentages of decrease were obtained. Interestingly, plasma α-tocopherol concentrations followed those of plasma TG and total cholesterol. The most important difference was between diets A and C (−59%); between diets A and B the difference was −39%, which was very close to the plasma TG and total cholesterol pattern. Consequently, when expressing the α-tocopherol data as the α-tocopherol to cholesterol plus TG ratio, no significant difference was found among the four dietary groups (Table 3). Plasma retinol concentration did not vary, except for a tendency to be lower when diet A was compared to diet C. Plasma GSH-PX remained fairly constant among the four groups, which suggests that the selenium status of rats was not altered by the diets.

### Heart and liver studies

When examining heart α-tocopherol concentration (**Fig. 1, top**), it is worthy of notice that increasing n-3 PUFA in diets led to a dramatically increased α-tocopherol concentration in this organ. It was 2.7-times higher in diet B than in diet A animals, and 4.8-times higher and 4.2-times higher in animals fed diets C and D, respectively. Total lipid phosphorus in heart was not significantly affected by the diets except for a tendency to be lower in diet D (data not shown). Consequently, when heart α-tocopherol concentration is expressed versus lipid phosphorus concentration, a similar pattern of heart α-tocopherol was obtained (Fig. 1, bottom). Conversely to heart, liver α-tocopherol concentration dramatically decreased as the n-6/n-3 decreased in the diet (Fig. 1,

TABLE 3. Plasma composition and plasma enzyme activities of rats fed various diets

| | Diet A | Diet B | Diet C | Diet D |
|---|---|---|---|---|
| | | *mean ± SD (n = 7)* | | |
| Proteins (g/l) | 68.9 ± 3.1 | 68.4 ± 2.2 | 69.0 ± 2.8 | 66.4 ± 2.6 |
| Albumin (μM) | 534 ± 21 | 536 ± 27 | 542 ± 31 | 510 ± 17 |
| Cholesterol (total g/l) | 2.25 ± 0.18$^{a**}$ | 1.55 ± 0.42$^{b}$ | 1.39 ± 0.18$^{b}$ | 1.12 ± 0.11$^{b}$ |
| Triglycerides (g/l) | 1.58 ± 0.33$^{a**}$ | 1.05 ± 0.25$^{b}$ | 0.87 ± 0.31$^{b}$ | 0.73 ± 0.18$^{b}$ |
| ASAT (μmol/min/l) | 146 ± 27.2 | 157 ± 27 | 154 ± 15 | 154 ± 26 |
| ALAT (μmol/min/l) | 49.2 ± 5.9 | 54.5 ± 6.8 | 55.5 ± 6.2 | 50.1 ± 6.2 |
| Alkaline phosphatase (μmol/min/l) | 179 ± 24$^{a*}$ | 208 ± 30$^{ab}$ | 167 ± 21$^{ab}$ | 228 ± 55$^{b}$ |
| GSH-PX (nmol/h/mg) | 100 ± 13 | 101 ± 11 | 110 ± 10 | 102 ± 9 |
| α-Tocopherol (mg/l) | 13.8 ± 1.26$^{a**}$ | 8.51 ± 2.44$^{b*}$ | 7.57 ± 1.24$^{bc}$ | 5.75 ± 0.91$^{c}$ |
| Vitamin A (mg/l) | 0.42 ± 0.05 | 0.43 ± 0.06 | 0.38 ± 0.09 | 0.35 ± 0.05 |
| α-Tocopherol/CH + TG (μmol/mmol) | 8.85 ± 0.72 | 8.07 ± 0.86 | 8.37 ± 1.1 | 7.56 ± 0.63 |

Values bearing different superscript letters are different at $P < 0.05^{*}$ or $P < 0.01^{**}$. When values bear different superscript letters and a different number of asterisks, the highest number has to be considered for the level of significance.

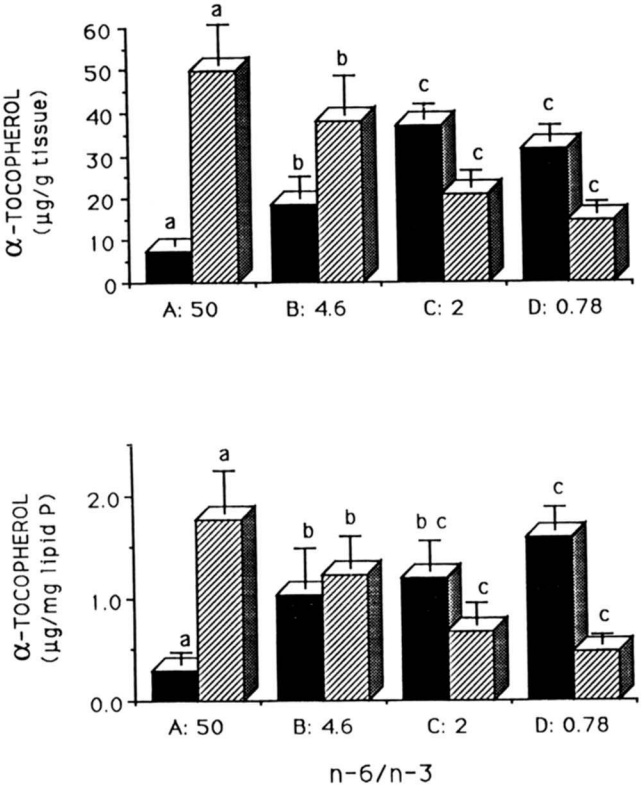


**Fig. 1.** Effect of dietary n-6/n-3 ratio on heart (black columns) and liver (hatched columns) α-tocopherol level expressed per g wet tissue (top) and per mg of total lipid phosphorus (bottom). For each tissue, column values not bearing the same superscript letter are statistically different at $P < 0.001$. Values are mean ± SD (n = 7).

top). Liver α-tocopherol concentration in rats fed diet D was about 3.4-times lower than in rats fed diet A. As for heart, when liver α-tocopherol concentration is expressed per g of lipid phosphorus, comparable variations were obtained (Fig.1, bottom). Liver γ-tocopherol was near the detection limit in all the dietary groups and thus did not exhibit any significant modification. The fatty acid composition of total lipids in heart (**Table 4**) and liver (**Table 5**) varied as a function of the nature of the PUFA added to the diet. That is to say that decreasing the dietary n-6/n-3 ratio resulted in a concomitant decrease in this ratio in tissue lipids and also in a higher index of unsaturation. The modifications of fatty acid composition we observed in total heart lipids mainly reflect those of membrane phospholipids since they represent about 90% of heart total lipids (8). These modifications are essentially due to the large increase in DHA and EPA, that occurred in diets B, C, and D (compared to diet A) at the expense of arachidonic acid. Thus, we plotted the heart α-tocopherol versus DHA or EPA contents or both (**Fig. 2**) and used a linear regression. We found a strong positive correlation between heart α-tocopherol and DHA contents ($r = 0.86$, $P = 0.0001$) whereas the correlation was less marked ($r = 0.65$, $P = 0.0003$) with EPA, but again highly significant with the sum of DHA and EPA ($r = 0.84$, $P = 0.0001$).

## DISCUSSION

In this study, we confirmed that, in rat, n-3 PUFA are more hypotriglyceridemic and hypocholesterolemic than n-6 PUFA (7, 8, 30, 31). The lipid-lowering effect of fish oil is certainly the result of a reduction of circulating lipoproteins, although not measured here. This can be achieved through either an accelerated removal or a decreased production of very-low-density lipoproteins by the liver (32–36). The most striking feature appearing in this study is the change in heart and liver α-tocopherol status when n-6 PUFA are replaced by n-3 PUFA.

TABLE 4. Fatty acid composition (weight %) of total lipids of hearts of rats fed various diets

| | Diet A | Diet B | Diet C | Diet D |
|---|---|---|---|---|
| | | *mean ± SD (n = 6)* | | |
| 16:0 | $10.8 \pm 0.8^{a**}$ | $11.4 \pm 0.8^{ab}$ | $12.8 \pm 1.2^{b}$ | $12.1 \pm 0.9^{ab}$ |
| 16:1 n-7 | $0.36 \pm 0.1^{a}$ | $0.54 \pm 0.0^{ab}$ | $0.63 \pm 0.1^{b*}$ | $1.01 \pm 0.24^{c**}$ |
| 18:0 | 22.6 ± 0.8 | 22.5 ± 2.4 | 24.4 ± 3.4 | 22.7 ± 1.9 |
| 18:1 n-9 | 5.93 ± 0.6 | 5.54 ± 1.1 | 5.36 ± 0.6 | 4.76 ± 0.4 |
| 18:1 n-7 | $3.28 \pm 0.2^{a*}$ | $3.00 \pm 0.2^{a**}$ | $3.15 \pm 0.3^{a**}$ | $3.69 \pm 0.1^{b}$ |
| 18:2 n-6 | $20.5 \pm 1.3^{a**}$ | $20.4 \pm 1.8^{a**}$ | $20.0 \pm 2.0^{a**}$ | $16.5 \pm 1.2^{b}$ |
| 20:2 n-6 | $0.34 \pm 0.02^{a}$ | $0.26 \pm 0.01^{ab*}$ | $0.15 \pm 0.01^{bc**}$ | $0.13 \pm 0.01^{c**}$ |
| 20:4 n-6 | $23.7 \pm 1.3^{a**}$ | $16.3 \pm 2.5^{b*}$ | $12.9 \pm 1.2^{c*}$ | $12.8 \pm 1.0^{c**}$ |
| 20:5 n-3 | $0.10 \pm 0.1^{a**}$ | $0.72 \pm 0.2^{b**}$ | $1.33 \pm 0.1^{c**}$ | $2.61 \pm 0.2^{d**}$ |
| 22:4 n-6 | $1.66 \pm 0.12^{a**}$ | $0.56 \pm 0.68^{b}$ | <0.1 | <0.1 |
| 22:5 n-3 | $1.28 \pm 0.1^{a}$ | $2.85 \pm 1.0^{b**}$ | $2.55 \pm 0.2^{b**}$ | $2.83 \pm 0.1^{b**}$ |
| 22:6 n-3 | $7.18 \pm 0.5^{a**}$ | $14.5 \pm 3.5^{b**}$ | $15.6 \pm 1.5^{b**}$ | $19.9 \pm 0.8^{c}$ |
| 24.1 n-9 | 0.22 ± 0.1 | 0.41 ± 0.4 | 0.15 ± 0.0 | 0.20 ± 0.1 |
| Others | 2.39 | 1.28 | 1.35 | 0.80 |
| n-6/n-3 | 5.4 | 2.1 | 1.7 | 1.2 |
| UI | 202 | 223 | 213 | 232 |

Values bearing different superscript letters are different at $P < 0.05$* or $P < 0.01$**. "Others" represents long chain saturated fatty acids. UI, unsaturation index, obtained by $\Sigma n \cdot x$, in which, for a given fatty acid, "n" is the number of double bonds and "x" is the weight percentage.

TABLE 5. Fatty acid composition (weight %) of total lipids of livers of rats fed various diets

| | Diet A | Diet B | Diet C | Diet D |
|---|---|---|---|---|
| | | *mean ± SD (n = 5)* | | |
| 16:0 | 21.4 ± 0.9 | 20.1 ± 2.3 | 19.4 ± 0.7 | 19.1 ± 1.3 |
| 16:1 n-7 | 1.65 ± 0.4$^{ab}$ | 1.26 ± 0.8$^{b*}$ | 1.77 ± 1.2$^{ab}$ | 2.96 ± 0.4$^{a**}$ |
| 18:0 | 8.82 ± 1.7 | 10.5 ± 1.9 | 10.2 ± 1.6 | 10.6 ± 2.3 |
| 18:1 n-9 | 19.0 ± 2.5$^{a}$ | 15.2 ± 1.0$^{b*}$ | 13.9 ± 1.4$^{b**}$ | 12.5 ± 1.3$^{b**}$ |
| 18:1 n-7 | 2.44 ± 0.1$^{a*}$ | 1.97 ± 0.2$^{b*}$ | 2.01 ± 0.2$^{b*}$ | 2.36 ± 0.2$^{ab}$ |
| 18:2 n-6 | 27.6 ± 1.8$^{a}$ | 24.0 ± 2.5$^{a}$ | 19.8 ± 1.4$^{b*}$ | 15.0 ± 1.0$^{c**}$ |
| 18:3 n-3 | 0.38 ± 0.03 | 0.45 ± 0.06 | 0.42 ± 0.06 | 0.45 ± 0.08 |
| 20:1 n-9 | 0.33 ± 0.03$^{a}$ | 0.57 ± 0.12$^{b*}$ | 0.77 ± 0.11$^{b**}$ | 1.02 ± 0.15$^{c**}$ |
| 20:4 n-6 | 12.8 ± 2.7$^{a}$ | 10.4 ± 0.7$^{ab}$ | 8.56 ± 1.5$^{b*}$ | 7.28 ± 1.2$^{b**}$ |
| 20:5 n-3 | <0.1$^{a**}$ | 3.23 ± 0.32$^{b**}$ | 4.54 ± 0.58$^{c**}$ | 8.03 ± 0.6$^{d**}$ |
| 22:4 n-6 | 0.90 ± 0.2$^{a**}$ | 0.21 ± 0.03$^{b}$ | 0.11 ± 0.03$^{b}$ | <0.1$^{b}$ |
| 22:5 n-6 | 0.49 ± 0.1$^{a**}$ | 0.10 ± 0.02$^{b}$ | 0.12 ± 0.02$^{b}$ | 0.17 ± 0.02$^{b}$ |
| 22:5 n-3 | 0.37 ± 0.1$^{a**}$ | 3.27 ± 0.8$^{b}$ | 3.68 ± 0.7$^{b}$ | 4.51 ± 1.2$^{b}$ |
| 22:6 n-3 | 2.58 ± 0.7$^{a**}$ | 10.2 ± 0.6$^{b**}$ | 13.6 ± 1.9$^{c**}$ | 15.4 ± 1.3$^{c**}$ |
| Others | 1.14 | 1.46 | 1.09 | 0.62 |
| n-6/n-3 | 12.2 | 2.02 | 1.30 | 0.79 |

Values bearing different superscript letters are different at $P < 0.05$* or $P < 0.01$**. "Others" represents long chain saturated fatty acids.

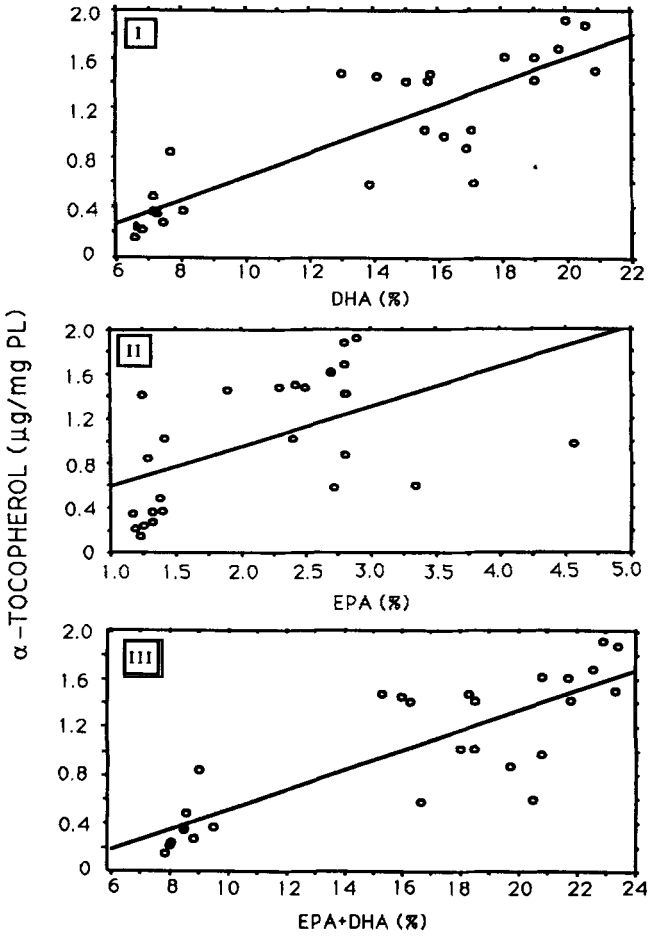


**Fig. 2.** Correlation between α-tocopherol concentration (μg/mg phospholipids (PL)) and DHA (I), EPA (II) and DHA + EPA (III) contents in hearts of rats fed various diets. Equations of the curves given by the computer were: A: y = −0.323 + 0.096x; $r$ = 0.86; $P$ = 0.0001; n = 27; B: y = −0.085 + 0.525x; $r$ = 0.65; $P$ = 0.0003; n = 26; C: y = −0.324 + 0.324x; $r$ = 0.84; $P$ = 0.0001; n = 27.

Previous works (7, 8) demonstrated *1*), by ultramicroscopic examination, an increase in lipid peroxide storage in myocytes when n-3 PUFA were administrated to rats and *2*) that the heart GSH-PX and GSH S-transferases were not altered by dietary n-3 PUFA (8). Thus, it was logical to expect a loss of α-tocopherol content in heart under comparable dietary treatment. In fact, heart α-tocopherol level was considerably increased together with an increase in membrane n-3 PUFA, mainly DHA. The increase in DHA and EPA content of rats fed n − 3 PUFA has been previously demonstrated in heart phosphatidylethanolamines and phosphatidylcholines (4, 5, 7, 8) which is confirmed here for total lipids that are about 90% represented by membrane phospholipids. The fact that we were able to observe such an increase strongly suggests that n-3 PUFA-enriched membranes are efficiently protected against peroxidation through the increased level of α-tocopherol.

The mechanism by which heart α-tocopherol content increased remains to be elucidated. It may be the consequence of an adaptive process developed by the heart to counterbalance a diet-induced higher sensitivity to membrane lipid peroxidation. This process could be achieved, for example, through an enhancement of the tocopherol binding protein (37, 38) that is thought to play a role in the intracellular transport of the vitamin in various organs, including heart (39). It has been shown that the activity of this protein can be modulated by the amount of dietary vitamin E (40). The fact that we did not observe a higher accumulation of intracytoplasmic lipid droplets in heart of rats fed a fish oil-enriched diet (8) allows us to suggest that the increase in heart α-tocopherol probably affected the membrane fraction. Thus, this increase could be the result of an alteration of the physicochemical pro-

perties of the membranes induced by incorporation of EPA and DHA. This would render them more able to accept α-tocopherol. The strong positive correlation that we found between heart α-tocopherol and DHA content suggests that the vitamin preferentially incorporated in DHA-enriched membrane. Interestingly, a close relationship has been found between α-tocopherol and PUFA contents of heart (41) and liver microsomes (41, 42), and liver mitochondria (42). Also, Traber et al. emphasized the role of lipoprotein lipase (43) and low-density lipoprotein receptor (44) in delivering α-tocopherol to tissues, a process that could be influenced by n-3 PUFA. It can however, be ruled out that the observed increase in heart α-tocopherol is due to a higher blood delivery since its plasma concentration was drastically reduced by n-3 PUFA intake. Liver is known to store (45) and specifically to secrete α-tocopherol via very-low-density lipoproteins (46) and more particularly the *RRR*-α-tocopherol stereoisomer (47, 48). This is indirectly confirmed herein since the diminution of plasma α-tocopherol closely paralleled that of plasma lipids. The drastic reduction of liver α-tocopherol content raises the question of which types of cells and subcellular compartments are affected by this depletion. Bjørneboe et al. (49) showed that liver parenchymal cells have storage capacity for α-tocopherol whereas non-parenchymal cells are more susceptible to α-tocopherol depletion. Liver mitochondria and microsomal fractions were demonstrated to contain most of the cellular α-tocopherol and 20% were found in the cytosol (42). The fact that plasma transaminase activities remained unchanged, independently of the diet, indicated that membrane injury through lipid peroxidation did not occur in the liver under our experimental conditions. Thus, it is possible that cytosolic intracellular stores were more affected by α-tocopherol depletion than membrane fractions. When considering the mass of liver, this 20% found in the cytosol (42) may represent a pool of α-tocopherol which is far from negligible to serve as a possible store for distribution to other organs. Nevertheless, this decline in α-tocopherol is probably a consequence of a higher incorporation of α-tocopherol in heart and perhaps other vital tissues such as brain or lung. Consequently, a lesser amount returns to the liver via the low-density lipoprotein receptor (44).

It remains, however, to be explained how lipofuscin accumulated in heart myocytes of rats fed (for 2 months) a diet with a n-6/n-3 ratio of 0.8 and a P/S ratio of 1.9 (7, 8). Except for the problem of duration of the feeding period, this may reflect an enhanced α-tocopherol activity in membrane. Indeed, the fatty acid hydroperoxides generated by vitamin E are released from membrane and then reduced by GSH-PX to prevent any subsequent decomposition into peroxyl radical, a reaction catalyzed by transition metals. Thus, it is possible that if higher amounts of fatty hydroperoxides are generated through the increase in α-tocopherol, some of these hydroperoxides may escape to GSH-PX reduction, thus leading to lipid peroxidation products. Another explanation is that the lipoperoxides originated from a source other than membrane phospholipids. For example, n-3 PUFA might have been peroxidized before being taken up by the heart, but directed toward lysosomes because they would be inappropriate for mitochondrial β-oxidation.

In conclusion, if the findings that we observed here with rat are extrapolable to human, several physiological implications should be considered. Among them, can the drastic alteration of α-tocopherol homeostasis affect the function of vital organs? Does the liver become more susceptible to lipid peroxidation under a peroxidative stress such as intoxication or chronic alcohol consumption (50), concomitantly with fish oil intake?

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