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DOCOSAHEXAENOIC ACID-ENRICHED EGG PHOSPHOLIPIDS SUPPLEMENTATION INDUCES ACCRETION OF ARACHIDONIC ACID IN RAT BLOOD LIPIDS

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Abstract - Animal and humans studies have shown that supplementation with triacylglycerides containing ω3 fatty acids, mainly docosahexaenoic acid (DHA) and eicosapentaenoic acid, can induce a decrease in arachidonic acid (AA) in blood lipids. Interestingly, we observed in a previous work that a supplementation with DHA enriched eggs in a healthy elderly population induced an accretion of AA in their blood lipids. The present study investigates whether purified DHA enriched egg phospholipids could be responsible for this effect. Four groups of rats were supplemented daily, for eight weeks, with DHA phospholipids (10, 30 or 60 mg/kg) or with soybean phospholipids. Red blood cell membranes and plasma fatty acid levels were compared with that of rats without supplementation. Soybean phospholipids supplementation increased the level of AA in blood lipids but decreased that of DHA. The doses of DHA phospholipids, 30 and 60 mg/kg, induced greater amounts of AA without affecting significantly DHA levels. In contrast, DHA phospholipids supplementation, 10 mg/kg, in which there was the greatest amount of AA, induced only a slight increase in AA levels. Moreover, DHA levels were decreased by this supplementation. These results demonstrate that specific increases in AA levels are preferentially associated with DHA phospholipids levels in supplementation.

Key words: Docosahexaenoic acid supplementation, glycerophospholipids, low doses, DHA/AA ratio, rats

INTRODUCTION

There are a number of observational and interventional studies revealing the beneficial effects on human development and health of ω3 fatty acids (FA) (9). Since these FA, as others, contribute to the acyl groups of glycerophospholipids, their presence is ubiquitous in tissues and numerous effects have been described from membrane protein activity (22) to cell functionality (20), lipid metabolism (8) and tissue (16), or even whole organism function (11). In addition, the ω3 FA family includes C18 to C22 FA that can have different and specific effects on lipid metabolism as observed in their interaction with the ω6 FA family (10), resulting in different physiological effects on blood pressure and heart rate (14). To add to the complexity, their bioavailability is not the same when given in the form of triacylglycerides or glycerophospholipids as observed for arachidonic acid (AA) (28) and docosahexaenoic acid (DHA) (12). A common trend is that increasing the ω3 FA content of the diet depresses the AA content of blood lipids (13,15,25,26). Conversely, in a previous study, we have observed that the supplementation of the diet of an elderly population with DHA enriched egg products, during a period of 9 months, resulted in a significant increase of AA levels in their blood lipids (19). Since the supplementation was based on egg yolk, a multi-components food, and that it is difficult to control such a population during a so long period of time, no conclusion was reached concerning the possible role of DHA. Here, we took advantage of the availability of pure glycerophospholipids, extracted from DHA enriched egg yolks, to confirm and extend on an animal model our former observations on institutionalized elderly. The influence of the quantity of these pure glycerophospholipid supplementation, as well as the value of the ratio of the ω6 to ω3 FA families, were investigated as possible mediators of the response of red blood cell (RBC) membrane and plasma FA composition to the diet.

Abbreviations: **AA**: arachidonic acid; **DHA**: docosahexaenoic acid; **EPA**: eicosapentaenoic acid; **FA**: fatty acids; **LC-PUFA**: long chain polyunsaturated fatty acids; **MUFA**: monounsaturated fatty acids; **PL**: phospholipids; **PUFA**: polyunsaturated fatty acids; **RBC**: red blood cell; **SB**: soybean; **SFA**: saturated fatty acids

MATERIAL AND METHODS

Animals

The study was conducted according to the guidelines of the French Ministry of Agriculture on the experimental use of laboratory animals (agreement number: A 13823). The principles of laboratory animal care (NIH) were followed. Male Sprague-Dawley rats, 6 to 7 weeks old (n = 50; Iffa Credo, Saint Germain de l'Arbresle, France) were entered in the study after acclimatization for one week. Their body weight at the beginning of the study averaged 233 ± 11 g, and they were randomly assigned to five age and weight-matched groups (n = 10). The animals had free access to food and water. The food was standard non-purified rodent diet (A04, UAR, Epinay sur Orge, France). One group was maintained on the standard diet (Control group) and the four other groups received, by gavage, a supplementation, once a day. Soybean phospholipids (S20, Lipoid, Ludwigshafen, Germany; 0.4 $g.kg^{-1}.day^{-1}$) were used as placebo (SB-PL group). DHA enriched-egg phospholipids were used at two dosages: 30 and 60 mg of $DHA.kg^{-1}.day^{-1}$ (LMA, Marseille, France; 0.4 and 0.8 $g.kg^{-1}.day^{-1}$) and regular egg phospholipids were used at one dose corresponding to 10 mg of $DHA.kg^{-1}.day^{-1}$ (commercial egg; 0.8 $g.kg^{-1}.day^{-1}$) (DHA-PL groups, Table 1). The soybean phospholipids contained by weight: lecithins derived from phospholipids, 43%; phosphatidylcholine, 21.5%; phosphatidyl-ethanolamine, 14.5%; phosphatidyl-inositol 12%; lysophosphatidylcholine, 3% and non-polar lipids, 3%. The egg phospholipids contained by weight: phosphatidylcholine 75%, phosphatidylethanolamine 20%, triacylglycerides, 2% and cholesterol 0.3%. The supplementation was given in the form of pre-swollen phospholipids (500 or 250 µl; 40% by weight in water).

Tissue preparations

After 8 weeks of supplementation, intracardiac blood was collected into sodium citrate-coated tubes. Plasma was separated by centrifugation at 1,500 g for 15 min. Leukocytes and platelets were removed from the blood samples by filtration through a micro-crystalline cellulose column (1). RBC were hemolyzed in 11 mM Tris buffer, centrifuged (30,000 g for 30 min at 4°C) and the membrane pellet was resuspended in 30 ml of buffer. The centrifugation step was repeated 3 times, as previously described (21). The washed RBC membranes and plasma were then stored at –80°C until extraction for fatty acid composition determination.

Plasma and RBC membrane fatty acid composition

Total lipids of plasma and RBC membranes were extracted with methanol and chloroform according to the method of Bligh & Dyer (2), we modified using a sonicator. Fatty acid composition was determined after methylation with BF3-methanol (Sigma, St. Louis, MO) according to Ohta *et al.* (17). The fatty acid methyl esters were analyzed by gas chromatography on a Perkin Elmer Autosystem XL (Perkin Elmer, Courtaboeuf, France) using a fused silica capillary column (25 m x 0.22 mm inner diameter), BPX 70, 0.25 µm (SGE, Villeneuve St. Georges, France) equipped with a flame ionization detector and the Turbochrom software. Hydrogen was used as the carrier gas. The temperature program ranged from 160°C to 205°C with a temperature rise of 1°C/min. Fatty acids were identified by their retention times on the column with respect to appropriate standards.

Statistical analysis

A Kolmogorov-Smirnov test for normality and a Bartlett test for homogeneous variance were performed for each group. All the data were investigated by a nonparametric Kruskal-Wallis test and differences between groups were identified by the Mann-Whitney U test. p-Values of less than 0.05 were considered significant. Regressions were considered significant for p-values of less than 0.05; R^2 and p were calculated with values from all rats in each group. All analyses were done by Statview software (Abacus Concepts, Berkeley, CA, USA) on Macintosh Ibook (Apple Computer, Les Ulis, France).

RESULTS

Food intake and weight gain were not significantly different between any of the dietary groups (data not shown).

Table 1 Fatty acid composition of diet supplementations

Fatty Acid ($mg.kg^{-1}.day^{-1}$)	Diets				
	Standard	+ SB-PL	+ DHA-PL10	+ DHA-PL30	+ DHA-PL60
C16:0	156	44.7	149	67.8	135.6
C18:0	30	14.3	103	40.7	81.4
C18:1	480	32.3	115	87.3	174.6
C18:2 ω6 (LA)	870	173.4	110	45.1	90.2
C18:3 ω3 (ALA)	Traces	21.6	0.5	/	/
C20:4 ω6 (AA)	/	/	43	10.6	21.2
C20:5 ω3 (EPA)	/	/	/	3.4	6.8
C22:6 ω3 (DHA)	/	/	10	30	60
Σ SFA	186	59	252	108.5	217
Σ MUFA	480	32.3	115	87.3	174.6
Σ PUFA	870	195	163.5	89.1	178.2
Σ ω6	870	173.4	153	55.7	111.4
Σ ω3	Traces	21.6	10.5	33.4	66.8
ω6/ω3	>>100	8.0	14.6	1.7	1.7
DHA/AA	/	/	0.2	2.8	2.8
ω6/ω3*	>>100	48	97	28	15

All groups received standard diet ± supplementations. AA: arachidonic acid; ALA: alpha linolenic acid; DHA: docosahexaenoic acid; DHA-PL: docosahexaenoic acid phospholipids 10, 30 and 60 $mg.kg^{-1}.day^{-1}$, respectively; EPA: eicosapentaenoic acid; LA: linoleic acid; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SB-PL: soybean phospholipids; SFA: saturated fatty acids; Σ: sum; * standard diet + supplementation.

Fatty acid composition of plasma lipids

Table 2 presents for the five regimens the average FA compositions of plasma lipids after 8 weeks. Compared to the standard diet, the SB-PL group shows a decrease in its DHA content and an increase in its linoleic acid (LA) content leading to increased polyunsaturated fatty acids

Table 2 Fatty acid composition of plasma lipids

Fatty Acid (% of total)	Diets				
	Standard	SB-PL	DHA-PL10	DHA-PL30	DHA-PL60
C16:0	19.6 ± 0.5^{a}	15.5 ± 0.4^{b}	17.8 ± 0.6^{c}	15.0 ± 0.7^{b}	14.9 ± 0.3^{b}
C16:1	3.5 ± 0.3^{a}	3.1 ± 0.4^{a,b}	2.6 ± 0.3^{a,b}	2.3 ± 0.4^{b}	2.5 ± 0.2^{b}
C18:0	6.9 ± 0.2^{a}	7.6 ± 0.4^{a,b}	7.5 ± 0.6^{a,b}	8.6 ± 0.5^{b}	8.0 ± 0.3^{b}
C18:1	15.1 ± 0.7^{a}	17.2 ± 1.4^{a,b}	17.7 ± 1.2^{a,b}	17.6 ± 1.7^{a,b}	19.3 ± 0.9^{b}
C18:2 ω6	19.9 ± 0.3^{a}	27.0 ± 1.1^{b}	19.6 ± 0.7^{a,c}	21.8 ± 0.6^{c}	21.8 ± 0.6^{c}
C20:3 ω6	0.8 ± 0.1^{a}	1.1 ± 0.1^{b}	1.4 ± 0.1^{b}	1.0 ± 0.1^{a,b}	1.3 ± 0.1^{b}
C20:4 ω6	13.1 ± 0.7^{a}	15.1 ± 1.1^{a,b}	14.7 ± 0.7^{a,b}	20.1 ± 2.4^{b}	16.6 ± 0.9^{b}
C20:5 ω3	1.7 ± 0.1^{a}	1.7 ± 0.2^{a}	1.3 ± 0.2^{a}	1.3 ± 0.1^{a}	1.5 ± 0.1^{a}
C22:5 ω3	1.9 ± 0.1^{a}	1.2 ± 0.1^{b}	1.9 ± 0.1^{a}	1.6 ± 0.1^{c}	1.6 ± 1.0^{c}
C22:6 ω3	5.4 ± 0.2^{a,c}	4.2 ± 0.1^{b}	5.0 ± 0.2^{a}	5.5 ± 0.2^{a,c}	6.3 ± 0.3^{c}
Σ SFA	26.5 ± 0.5^{a}	23.1 ± 0.5^{b,c}	25.3 ± 0.6^{b}	23.6 ± 0.5^{b,c}	22.9 ± 0.4^{c}
Σ MUFA	18.6 ± 1.0^{a}	20.3 ± 1.8^{a,b}	20.3 ± 1.5^{a,b}	19.9 ± 2.0^{a,b}	21.8 ± 1.0^{b}
Σ PUFA	42.8 ± 1.2^{a}	50.3 ± 1.8^{b}	44.9 ± 0.7^{a}	51.3 ± 2.6^{b}	49.1 ± 0.9^{b}
Σ ω6	33.8 ± 0.9^{a}	43.2 ± 1.9^{b}	35.7 ± 0.6^{a}	42.9 ± 2.5^{b}	39.7 ± 1.0^{b}
Σ ω3	9.0 ± 0.4^{a,c}	7.1 ± 0.3^{b}	8.2 ± 0.2^{a}	8.4 ± 0.3^{a}	9.4 ± 0.2^{c}
ω6/ω3	3.8 ± 0.1^{a}	6.2 ± 0.5^{b}	4.4 ± 0.1^{c,d}	5.2 ± 0.3^{c}	4.2 ± 0.2^{d}
Σ LC-PUFA	22.9 ± 1.0^{a}	23.3 ± 1.0^{a}	24.3 ± 1.0^{a}	29.5 ± 2.6^{a,b}	27.3 ± 0.8^{b}
DHA/AA	0.42 ± 0.1^{a}	0.29 ± 0.1^{b}	0.34 ± 0.1^{a,b}	0.30 ± 0.1^{b}	0.38 ± 0.1^{a}

Values are means ± SEM for n = 10. Values not sharing at least one same superscript are significantly different with $p<0.05$. AA: arachidonic acid; DHA: docosahexaenoic acid; DHA-PL: docosahexaenoic acid phospholipids 10, 30 and 60 $mg.kg^{-1}.day^{-1}$, respectively; LC-PUFA: long chain-polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SB-PL: soybean phospholipids; SFA: saturated fatty acids; Σ: sum

Table 3 Fatty acid composition of membrane erythrocyte

Fatty Acid (% of total)	Diets				
	Standard	SB-PL	DHA-PL10	DHA-PL30	DHA-PL60
C16:0	25.7 ± 0.3^{a}	22.5 ± 0.6^{b}	23.2 ± 1.0^{b}	20.4 ± 0.2^{c}	21.4 ± 0.4^{b}
C18:0	14.1 ± 0.2^{a,c}	16.3 ± 0.3^{b}	13.2 ± 0.4^{a}	14.9 ± 0.3^{c}	15.3 ± 0.5^{a,b,c}
C18:1	9.9 ± 0.1^{a}	11.0 ± 0.4^{a,c}	9.2 ± 0.2^{b}	10.4 ± 0.3^{a,c}	11.2 ± 0.3^{c}
C18:2 ω6	9.1 ± 0.2^{a}	11.3 ± 0.4^{b}	8.8 ± 0.2^{a}	9.9 ± 0.2^{c}	10.4 ± 0.3^{c}
C20:4 ω6	18.4 ± 0.8^{a}	23.0 ± 0.7^{b}	20.3 ± 1.1^{a,b}	27.3 ± 0.1^{c}	25.4 ± 0.7^{d}
C20:5 ω3	1.7 ± 0.1^{a}	0.9 ± 0.1^{b}	1.4 ± 0.3^{a,b}	0.7 ± 0.1^{b}	0.9 ± 0.1^{b}
C22:5 ω3	3.1 ± 0.2^{a}	1.9 ± 0.1^{b}	3.8 ± 0.2^{a}	2.7 ± 0.1^{c}	2.5 ± 0.1^{d}
C22:6 ω3	5.3 ± 0.3^{a}	3.1 ± 0.2^{b}	4.2 ± 0.2^{c}	5.6 ± 0.1^{a}	5.2 ± 0.2^{a}
Σ SFA	39.8 ± 0.4^{a}	38.8 ± 0.6^{a}	36.4 ± 1.2^{b}	35.3 ± 0.3^{b}	36.7 ± 0.7^{b}
Σ MUFA	9.9 ± 0.1^{a}	11.0 ± 0.4^{a,c}	9.2 ± 0.2^{b}	10.4 ± 0.3^{a,c}	11.2 ± 0.3^{c}
Σ PUFA	37.6 ± 1.0^{a}	40.2 ± 0.8^{b}	38.5 ± 1.2^{a}	46.2 ± 0.2^{c}	44.4 ± 0.9^{d}
Σ ω6	27.5 ± 1.0^{a}	34.3 ± 0.6^{b}	29.1 ± 1.3^{a}	37.2 ± 0.2^{c}	35.8 ± 0.8^{b,c}
Σ ω3	10.1 ± 0.3^{a}	5.9 ± 0.3^{b}	9.4 ± 0.2^{a,c}	9.0 ± 0.1^{c,d}	8.6 ± 0.2^{d}
ω6/ω3	2.8 ± 0.1^{a}	5.7 ± 0.3^{b}	3.1 ± 0.2^{a}	4.1 ± 0.1^{c}	4.2 ± 0.1^{c}
Σ LC-PUFA	28.5 ± 0.8^{a}	28.9 ± 0.9^{a}	29.7 ± 1.1^{a}	36.3 ± 0.1^{b}	34.0 ± 0.8^{c}
DHA/AA	0.29 ± 0.1^{a}	0.14 ± 0.1^{b}	0.21 ± 0.1^{c}	0.20 ± 0.1^{c}	0.21 ± 0.1^{c}

Values are means ± SEM for n = 10. Values not sharing at least one same superscript are significantly different with $p<0.05$. AA: arachidonic acid; DHA: docosahexaenoic acid; DHA-PL: docosahexaenoic acid phospholipids 10, 30 and 60 $mg.kg^{-1}.day^{-1}$, respectively; LC-PUFA: long chain-polyunsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SB-PL: soybean phospholipids; SFA: saturated fatty acids; Σ: sum

(PUFA) without change of long-chain PUFA (LC-PUFA) contents. In the case of the 10 DHA-PL group, the only significant change concerns the di-homo gamma linolenic acid (+60%) and the total content (-6%) in saturated FA (SFA). Groups 30 and 60 DHA-PL show higher AA contents (+53% and +27%, respectively), the 60 DHA-PL was the sole supplementation group for which an increase of DHA was observed (+17%, not significant p = 0.06), all together this lead to increased PUFA (+20% and +15%, respectively) and LC-PUFA contents (+28% and +19%, respectively) for these two groups.

Fatty acid composition of RBC membrane lipids

Table 3 presents the average FA compositions of RBC membrane lipids. Compared to the standard diet, the SB-PL group presents a decrease in its DHA content and increases in LA, in AA (+25%) and PUFA contents with no significant change in LC-PUFA. The ratio DHA/AA is reduced by a factor two. For the 10 DHA-PL group, a decrease in DHA content is accompanied by that of the sum of the SFA. The 30 and 60 DHA-PL groups show no significant change in DHA but increased their AA content (+48% and +38%, respectively) while beside a slight decrease in the SFA content corresponds an increase in PUFA (+22% and +18%, respectively), which reflects the behavior of the LC-PUFA content (+28% and +20%, respectively). For the three DHA-PL groups, the ratio DHA/AA is reduced to a rather constant value (0.205 ± 0.002) intermediate between those calculated for the control and the SB-PL groups (0.29 and 0.14, respectively).

Relation between the AA content of the diet and its variation in the blood lipids

Fig. 1 presents the relation between the daily amounts of AA, in mg per kg of rat, brought by the supplementation in the case of DHA-PL groups, and the increases in AA percentage in RBC membrane FA relative to that measured in the case of the control group. The data fit to a straight line of equation: ($y = -0.21x + 8.81$, $R^2 = 0.16$, $p = 0.05$) in the case of plasma lipids and to a line of equation: ($y = -0.30x + 12.60$, $R^2 = 0.74$, $p<0.0001$) in the case of RBC membrane FA. Both show a negative slope.

Relation between the relative increase of linoleic acid in the diet and the AA increase in the blood lipids

Fig. 2 presents the relation between the percent increase of LA in the diet following the DHA supplementations and the corresponding increases in AA percentages in plasma and RBC membrane FA compared to that measured in the case of the control group. The data fit to a straight line of equation: ($y = -0.88x+14.06$, $R^2 = 0.55$, $p<0.0001$) for RBC membrane FA, and ($y = -0.71x+10.73$, $R^2 = 0.21$, $p = 0.03$) for plasma lipids, both are characterized by their negative slope.

DISCUSSION

Our study evidences a regulatory role of DHA enriched phospholipids, i.e. 30/60 DHA-PL, in the diet on fatty acid composition of plasma and RBC membranes and particularly on AA levels. Furthermore, we demonstrate that AA levels accretion in blood lipids correlate negatively with both AA and LA levels in diet.

The data presented here concern four supplementations in fatty acids of the ω6 and ω3 FA families brought in the form of purified glycerophospholipids to the diet of adult rats. They represent an increase in the daily fatty acid content of the diet of 19% for SB-PL and DHA-PL30 groups, and 37%

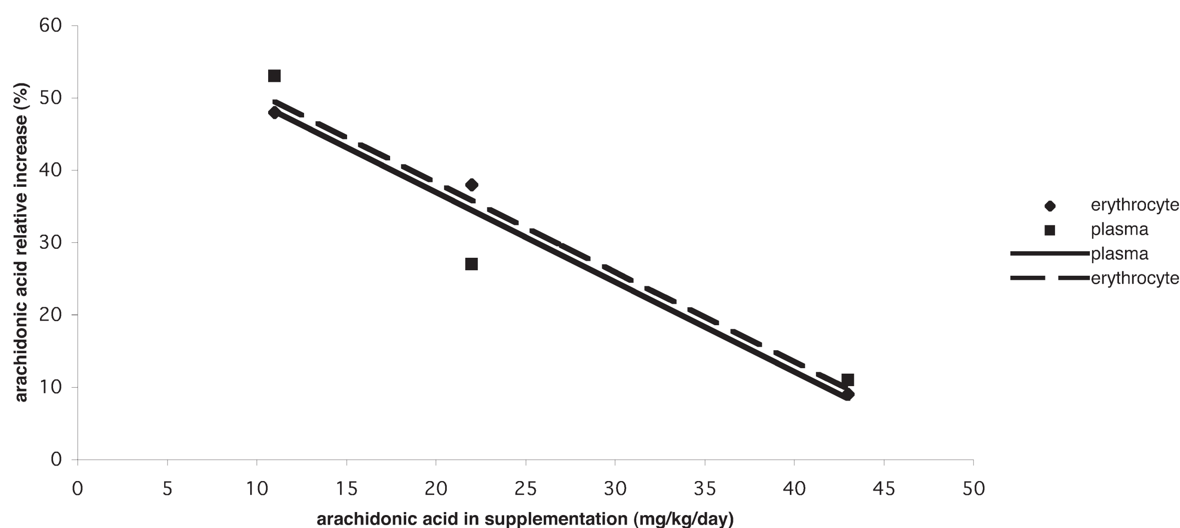

Fig. 1 Relation between the AA content of the diet and its variation in the blood lipids. Negative correlations between arachidonic acid in supplementation and arachidonic acid relative increase in plasma lipids ($R^2 = 0.16$, $p = 0.0471$) or in RBC membrane FA ($R^2 = 0.74$, $p<0.0001$) are observed.

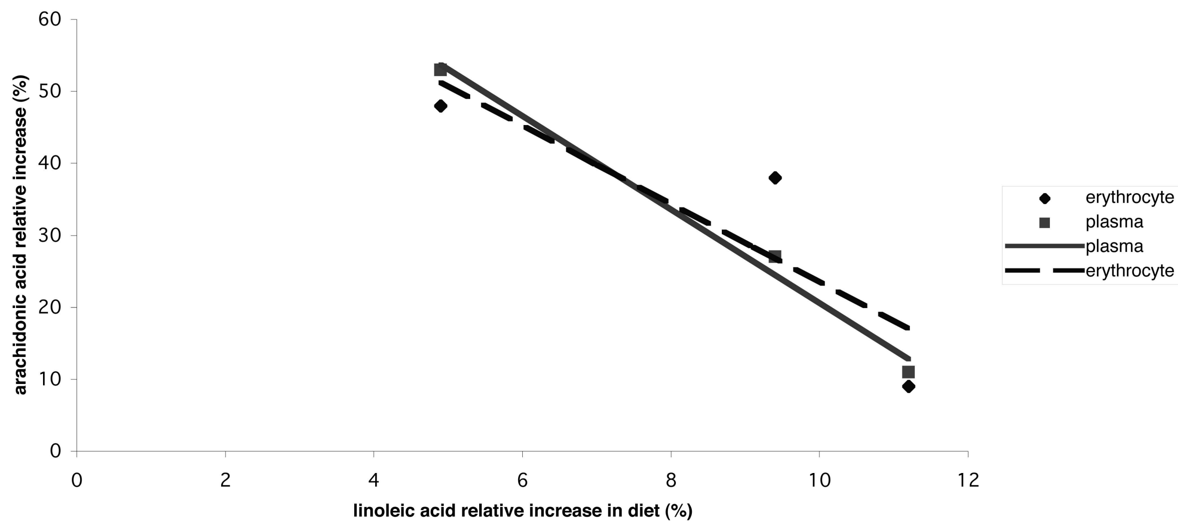

Fig. 2 Relation between the relative increase of linoleic acid in the diet and the AA increase in the blood lipids. Negative correlations between linoleic acid relative increase in diet and arachidonic acid relative increase in plasma lipids (R^2 = 0.21, p = 0.0259) or in RBC membrane FA (R^2 = 0.55, p<0.0001) are observed.

for DHA-PL10/60 groups, corresponding to a very low caloric increase of the diet (0.7% and 1.4%, respectively). The standard chow used all along this study brought very likely ω3 LC-PUFA. This chow contains 2% by weight of fishmeal in which it usually remains 10 to 20% of fish oil rich in ω3 LC-PUFA. This may explain why in the case of the standard diet the DHA/AA ratio in plasma (0.42) is already high. Nevertheless, it is important to note that the balance between the C18 ω6 and C18 ω3 FA was not at all equilibrated since the ratio ω6/ω3 was well over 100 (Table 1). Following the supplementations, the ω6/ω3 ratio decreased in the diet to a value of 48 for the SB-PL group and to 97, 28 and 15 for the three DHA-PL groups, respectively. The ω6/ω3 ratio was corrected by alpha linolenic acid in the case of the SB-PL supplementation while in the case of the DHA-PL groups, DHA and eicosapentaenoic acid (EPA) were the main contributors to its reduction. These latter groups brought, in addition to DHA, AA at two different ratios (DHA/AA = 0.2 and 2.8).

In most of the situations following a supplementation with DHA and EPA, the literature reports a decrease in AA levels in blood lipids (15,25,26). Here, the decrease in the ω6/ω3 ratio in the diet, due to increased amounts of ω3 FA, surprisingly corresponds in each case, to an increase in the AA contents of plasma and RBC membrane FA. This increase was found significant and it reaches +48% and +38% in the case of RBC for 30 and 60 DHA-PL groups, respectively. This higher content in AA does not result only from dietary origin since Fig. 1 clearly shows that a negative correlation exists between the quantities of dietary AA brought by the supplementation and its percent increase in the plasma and RBC membrane FA. It is worth to mention that a comparable AA accretion in rat brain lipids following DHA enriched egg phospholipids supplementation was recently reported. Indeed, a negative correlation was observed between the amount of AA supplied by the diet and the increase in the proportion of AA, which does not support a direct dietary origin (6). In fact, it has been reported an accretion of AA in liver proportionally related to its dietary levels when the diet contains 50-100 times more AA than our study (27). Hence, in our study, such increase would result from a modification of the FA metabolism. Two main options can be considered: a) an increased biosynthesis or/and, b) a reduced catabolism of AA. The data of Fig. 2 show that the increase in AA does not solely result from the diet content in linoleic acid, its precursor, since a negative correlation was observed. These data support the conclusion that the biosynthetic pathway was not very active since the quantity/proportion of AA synthesized does not respond to increased amounts of its precursor. Whelan *et al.* (27) have already shown that the doubling of dietary linoleic acid resulted in no significant increase in liver AA level. The most likely interpretation is that the increased proportions of AA observed in the case of the DHA-PL supplementations resulted from a reduced catabolism of AA. Such an interpretation can be related to the already observed effects of a low intake of ω3 FA (180 mg/day) in elderly people which decreased the oxidative stress in platelets likely due to a lower cell peroxide level (24). On the opposite, high intakes (5 g/day) of ω3 FA given in the form of fish oil increased the requirement for dietary antioxidants (18).

Furthermore, the variations in FA compositions lead to fairly constant values of the DHA/AA ratio in the case of RBC membrane FA, 0.205 ± 0.002 for the three DHA-PL

groups. This can be the sign of a strong regulatory action of these supplementations. In addition, these variations in FA compositions resulted in higher proportions of PUFA and LC-PUFA. The fact that the higher increases in AA were calculated for the 30 DHA-PL supplementation supports the idea that the 60 DHA-PL supplementation may correspond to an excess dose. Hence, even if these two supplementations gave the same DHA/AA ratio, the higher dosage appears less efficient. This might support the idea that one may have to deal with bell-shaped dose-response curve like it has been reported for the oxidative status in response to ω3 FA supplementation (18,24).

It is worth to mention that comparable observations were noted when an elderly population was supplemented with DHA enriched egg products. Indeed, the percentages of AA increased in RBC membranes like the proportion of PUFA and LC-PUFA. Also, a regulatory action on the fatty acid metabolism was evidenced through the reduction of the values of the standard deviations associated with the FA proportions (19). Hence, these data confirm the former observations made on an elderly population and support the conclusion that the dietary DHA enriched phospholipids are the cause of important modifications of the FA metabolism as reflected by the changes in FA composition of blood lipids.

It is of importance that this supplementation, bringing DHA, can increase AA levels in membranes. Indeed, DHA and AA have important regulatory effects on the structure and physical properties of membrane because they modulate receptors affinities and enzyme activities such as Na,K-ATPase. A positive correlation has been shown between the levels of ω6 fatty acids in some tissue membranes and the affinity of the Na,K-ATPase for ouabain (7). Moreover, diabetes decreases the activity of this enzyme in different tissues affected by complications (3,4,23). So, the use of polyunsaturated fatty acid supplementations can be of help to restore the Na,K-ATPase activity for minimizing the severity of these complications (3,5). Like chronic diseases are frequently associated with abnormal levels of PUFA in membranes, this DHA-phospholipids supplementation, not reducing AA levels, can provide important health benefits.

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