Dietary *n*-9, *n*-6 and *n*-3 fatty acids modify linoleic acid more than arachidonic acid levels in plasma and platelet lipids and minimally affect platelet thromboxane formation in the rabbit

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We have studied the effects of semisynthetic diets containing 5% by weight (12% of the energy) of either olive oil (70% oleic acid, OA) or corn oil (58% linoleic acid), or fish oil (Max EPA, containing about 30% eicosapentaenoic, EPA C 20:5 n-3, plus docosahexaenoic, DHA C 22:6 n-3, acids, and less than 2% linoleic acid), fed to male rabbits for a period of five weeks, on plasma and platelet fatty acids and platelet thromboxane formation. Aim of the study was to quantitate the absolute changes of n-6 and n-3 fatty acid levels in plasma and platelet lipid pools after dietary manipulations and to correlate the effects on eicosanoid-precursor fatty acids with those on platelet thromboxane formation. The major differences were found when comparing the group fed fish oil and depleted linoleic acid vs the other groups. The accumulation of n-3 fatty acids in various lipid classes was associated with modifications in the distribution of linoleic acid and arachidonic acid in different lipid pools. In platelets, maximal incorporation of n-3 fatty acids occurred in phosphatidyl ethanolamine, which also participated in most of the total arachidonic acid reduction occurring in platelets, and linoleic acid, more than archidonic acid, was replaced by n-3 fatty acids in various phospholipids. The archidonic acid content of phosphatidyl choline was unaffected and that of phosphatidyl inositol only marginally reduced. Thromboxane formation by thrombin stimulated platelets did not differ among the three groups, and this may be related to the minimal changes of arachidonic acid in phosphatidyl choline and phosphatidyl inositol.

Keywords: fish oil, corn oil, olive oil, plasma fatty acids, platelet fatty acids, and thromboxane

Introduction

Dietary fatty acids modify the composition of plasma and tissue lipids, with subsequent changes of biochemical and functional parameters in various biological compartments. The amounts and proportions of saturated and polyunsaturated fatty acids (PUFA) of the *n*-6 and *n*-3 series, for instance, influence plasma cholesterol levels and affect the aggregation of platelets[1,2] through modifications of the eicosanoid cascade.[3-5]

The administration of PUFA of either the *n*-6 series, such as linoleic acid (LA), or of the *n*-3 series, such as eicosapentaenoic acid (EPA, 20:5 *n*-3) results in modifications of the 20 carbon eicosanoid-precursors in cell lipids[6] and in subsequent quantitative and qualitative changes of eicosanoid production.[5,7] This paper addresses the issue of establishing the absolute changes of the major *n*-6 and *n*-3 PUFA in plasma and platelet lipids, after administration of diets rich in *n*-9, *n*-6, and *n*-3 fatty acids, respectively, and of relating dietary induced changes of platelet fatty acids with the formation of TxB_2 in rabbits. The rabbit was selected as experimental animal for the large volume of blood that can be drawn, allowing the preparation of platelet samples of adequate size for quantitative measure-

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Received November 8, 1989; accepted for publication May 31, 1990.

ments of fatty acid pools from a single animal. In addition, this species is widely used in studies on experimental thrombosis and atherosclerosis and, thus, is quite suitable for investigating the effects of dietary fats on these parameters. The animals were treated according to the approval of our Institutional Animal Care Review Board.

Our data indicate that in the rabbit, administration of a diet containing fish oil (FO) as the only fat results in selected replacement of LA, rather than arachidonic acid (AA), by *n*-3 fatty acids, and this effect is not associated with a significant modification of platelet thromboxane formation.

Materials and methods

Animals and dietary treatments

Three groups of New Zealand male rabbits (of ten animals each, of the average weight of 2.5 kg) were fed *ad libitum* semisynthetic diets (Piccioni, Agrate Brianza, Italy) with adequate protein, carbohydrate, vitamin, and salt contents, and containing 5% by weight (12% of the energy) as either olive oil (OO), or corn oil (CO) or fish oil (FO, MaxEPA, Seven Seas, U.K.) for a period of five weeks. The amount of fat was comparable to that present in standard diets. The diets were supplemented with α tocopherol in the amount of 50 mg/kg of diet. The fatty acid composition of dietary fat is presented in Table 1.

Diets were stored at −20° and fresh diets were given every day to the animals. The intakes of individual fatty acids, calculated on the basis of the daily consumption of diets and of the body weight (bw), in the three groups of animals were, on the average, the following: oleic acid (OA) in the OO group 1.2 g/Kg bw (8.4% of the energy), LA in the CO group 1.3 g/Kg bw (7.0% of the energy), EPA + DHA in the FO group 0.55 g/Kg bw (3.6% of the energy).

Blood collection

At the end of the five week dietary treatments, after an overnight fast, animals were anaesthetized with Na thiopenthal (50 mg/kg) and blood (up to 100 ml/animal) was collected from cannulated common carotid arteries. Blood was drawn in large plastic tubes using ACD 15% vol/vol (ACD, citric acid 71 mM; Na citrate 85 mM; dextrose 111 mM) as anticoagulant. After collection of blood animals were sacrificed.

Table 1 Fatty acid percentage composition of dietary fats

Fatty acids	OO	CO	FO
14:0	—	—	8.3
16:0	17.1	13.3	17.6
16:1	—	—	8.7
18:0	—	1.9	2.6
18:1	70.1	26.6	14.4
18:2	12.8	58.1	1.7
20:0	—	—	3.5
20:5	—	—	15.8
22:1	—	—	5.0
22:5	—	—	2.0
22:6	—	—	14.0

OO, olive oil.
CO, corn oil.
FO, fish oil.

Preparation of platelet and plasma samples

Blood drawn from one animal was subdivided in aliquots in plastic tubes and platelet rich plasma (PRP) was obtained by low speed centrifugation (150 × g for 18 min) and the platelet pellet was resuspended in modified Tyrode-HEPES buffer (134 mM NaCl, 12 mM $NaHCO_3$, 0.34 mM Na_2HPO_4, 1 mM $MgCL_2$, 5 mM HEPES, 5 mM glucose, 1 mM EGTA) pH 7.4, containing PGI_2 (5 ng/ml). Preparation of plasma samples for lipid analysis from blood aliquots anticoagulated with ACD was carried out by centrifugation of PRP and resuspension in Tyrode-HEPES buffer.

Preparation of washed platelets for lipid analysis was performed by centrifuging the platelet suspensions and subjecting the samples to osmotic shock in distilled water and to a final high speed centrifugation.

Lipid analysis

Lipids were extracted from plasma and from washed platelets with chloroform/methanol 2:1, containing 5 μg/ml of the antioxidant butylated hydroxytoluene (BHT). The total lipid contents of the extracts were quantified by micro gravimetric procedures. Separation of major plasma lipid classes was achieved by one dimensional thin layer chromatography, using hexane/diethyl ether/acetic acid 70:30:2 as developing solvent on Silica Gel HR 60 (Merck, 250 μm thickness) plates. Separation of platelet phospholipid classes was carried out by two dimensional thin layer chromatography on plates spread with Silica Gel HR (Merck) containing 7.6% (wt/wt) Mg acetate and using chloroform/methanol/NH_4OH 65:35:5 in the first dimension and chloroform /acetone/methanol/acetic acid/water 3:4:1:1:0.5 as developing solvent, in the second. Fatty acid methyl esters of separated lipids were prepared by transmethylation using methanolic HCl (Supelco, Bellofonte, PA) and analyzed by GLC on capillary columns (Supelcowax 10.30 m, 0.75 mm i.d., 1.0 μm df) and programmed temperature (140° to 210° at 2.5°/min increments). Quantitation of fatty acids was carried out by the use of C 19:0 added to the lipid extract as internal standard, and calibration curves were obtained by plotting reference fatty acids vs the internal standard after GLC determination. Statistical analysis for comparisons of data among groups was carried out by the Dunnett test.[8,9]

Thromboxane formation by stimulated platelets

Platelet thromboxane formation was evaluated by measuring the levels of TxB_2 in PRP from the different animal groups at 2 min following stimulation with increasing concentrations of thrombin. TxB_2 determination was carried out by a specific RIA procedure.[10]

Results

Plasma fatty acids

The percentage compositions of major fatty acids of plasma lipids (cholesterol esters, CE, triacylglycerols, TG, and phospholipids, PL) from the three groups of animals are shown in Table 2. The fatty acid profiles generally reflected the different dietary intakes. OA was, thus, significantly higher in plasma lipids of the OO group, LA was elevated in lipids of the CO group and EPA and DHA were elevated in the FO group.

Analysis of differences among groups in each lipid fraction revealed that in PL, OA levels were highest in the OO and lowest in the FO group, whereas LA levels were highest in CO and lowest in FO groups. As expected, *n*-3 fatty acids were present only in the FO group, with maximal increment for DHA.

Differences among groups in CE followed a trend similar to that described for PL, but in the FO group LA levels were not much lower than in the OO group, and levels of EPA were greater than those of DHA. In TG, differences among groups for OA and LA were comparable to those observed in CE, and in the FO group, levels of *n*-3 fatty acids showed a pattern similar to that of the administered oil.

Table 2 Fatty acid percentage composition of plasma phospholipids (PL), cholesterol esters (CE) and triglycerides (TG)

Fatty acids	Dietary fats		
	OO	CO	FO
	Phospholipids		
16:0	23.5 ± 0.9[d]	26.5 ± 1.6	32.0 ± 0.6[b,d]
18:0	22.2 ± 1.2	23.2 ± 0.9	21.0 ± 0.6
18:1	19.5 ± 2.3[c,d]	10.3 ± 1.1[a,c]	7.2 ± 0.6
18:2 ω6	29.3 ± 1.5[d]	33.4 ± 3.2[d,†]	7.5 ± 1.0[d,d,†]
20:4 ω6	5.3 ± 0.5[a]	6.5 ± 0.7	7.2 ± 0.4[a]
20:5 ω6	—	—	7.6 ± 1.1
22:5 ω6	—	—	4.7 ± 0.3
22:6 ω6	—	—	12.8 ± 0.7
	Cholesterol esters		
16:9	18.4 ± 1.5[d]	18.5 ± 0.9[e]	37.0 ± 1.3[b,d]
18:0	4.7 ± 0.4	3.4 ± 0.4	4.1 ± 0.3
18:1	49.6 ± 1.8[d]	30.0 ± 1.2[d]	22.1 ± 0.6[d]
18:2 ω6	26.0 ± 1.1[d]	49.0 ± 2.3[d,d,†]	20.2 ± 2.5[d,c,†]
20:4 ω6	1.4 ± 0.1[d]	2.2 ± 0.2[d,d,†]	4.5 ± 0.3[d,†]
20:5 ω6	—	—	9.0 ± 1.4
22:5 ω6	—	—	0.2 ± 0.1
22:6 ω6	—	—	3.4 ± 0.6
	Triglycerides		
16:0	38.0 ± 1.7[c]	37.0 ± 1.3[c,†]	28.3 ± 2.1[c,c,†]
18:0	4.6 ± 0.5	4.2 ± 0.4	4.6 ± 0.8
18:1	41.0 ± 2.2[c,d]	29.2 ± 1.5[c,d,†]	16.7 ± 0.9[d,d,†]
18:2 ω6	16.2 ± 1.6[d]	30.8 ± 2.1[d]	16.5 ± 1.2[d,†]
20:4 ω6	0.5 ± 0.1[d]	0.8 ± 0.2[d,†]	3.0 ± 0.3[d,d,†]
20:5 ω6	—	—	14.7 ± 2.4
22:5 ω6	—	—	6.0 ± 0.9
22:6 ω6	—	—	10.1 ± 1.2

Values are the average ± SEM of six measurements. Values sharing the same superscript are significantly different from each other.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.005$.
[d] $p < 0.001$.

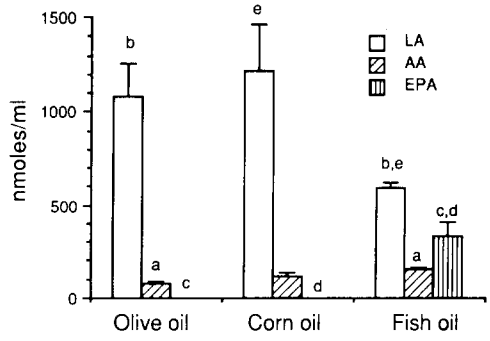

Figure 1 Levels (nmoles/ml) of linoleic (LA), arachidonic (AA), and eicosapentaenoic (EPA) acids in plasma of rabbits fed three different oils. Values (average ± SEM) with the same letter are statistically different from each other at the following levels: a: $p < 0.0125$; b,e: $p < 0.024$; c,d: $p < 0.005$.

The absolute levels (nmoles/ml) of LA, AA, and EPA, selectively measured in plasma, are shown in Fig. 1. Levels of LA were tenfold greater than those of AA in the OO and CO groups, whereas in the FO group the ratios of LA to AA and EPA were about one to one. It is of interest that the absolute levels of AA in plasma of the FO group were not reduced with respect to the CO and OO groups. In the FO group levels of EPA exceeded those of AA. Absolute levels of palmitic acids were 1,277 ± 81 nmoles/ml plasma in the FO group and 1,000 ± 67 and 1,093 ± 89 nmoles/ml, respectively, in the CO and OO groups. These differences are not statistically significant.

The distributions of LA, AA, and EPA among the major plasma lipid classes are presented in Figs. 2 and 3. Most of plasma LA was associated with the PL fraction in the OO and CO groups (Fig. 2, panel A). In the FO group, instead, plasma LA was mainly associated with the TG fraction. The distribution of AA among lipid classes (Fig. 2, panel B) was very similar in the OO and CO groups, with about 80% of this fatty acid associated with the PL fraction, but in the FO group AA was reduced in PL and enhanced in both TG and CE, with respect to the OO and CO groups. In the FO fed animals (Fig. 3), AA was still predominantly associated with the PL fraction, whereas EPA was mainly associated with the TG fraction.

Platelet fatty acids

The fatty acid composition of platelet lipids reflected the fatty acid pattern of plasma lipids. The absolute levels of fatty acids in individual lipid classes, however, were differentially affected by dietary manipulations. Levels (nmoles/mg of platelet total lipid) of the selected polyunsaturated fatty acids LA, AA, and EPA in total lipids from platelets of rabbits fed different oils are shown in Fig. 4. LA levels were highest in the CO group and lowest in the FO group, AA was practically identical in the OO and CO groups and slightly, although significantly, reduced in the FO

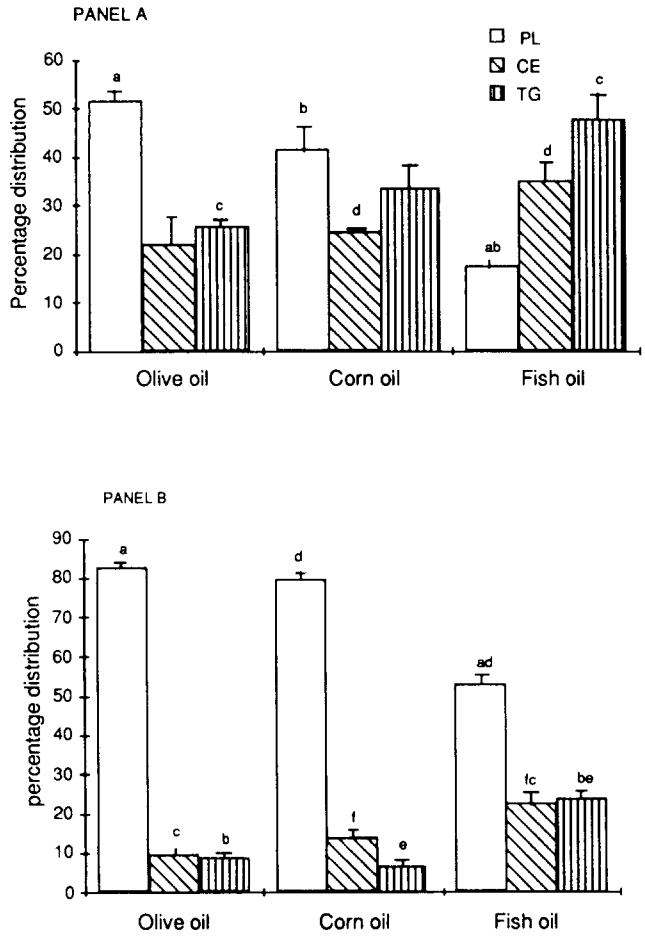

Figure 2 Percentage distribution (average ± SEM) of linoleic acid (Panel A) and arachidonic acid (Panel B) among the major plasma lipid classes in rabbits fed three different oils. Values with the same letter are statistically different from each other at the following levels: Panel A = a:$p < 0.0005$; b:$p < 0.0025$; c:$p < 0.01$; d:$p < 0.0125$. Panel B = a,b:$p < 0.0005$; c:$p < 0.01$; d:$p < 0.0025$; e:$p < 0.005$; f:$p < 0.0125$.

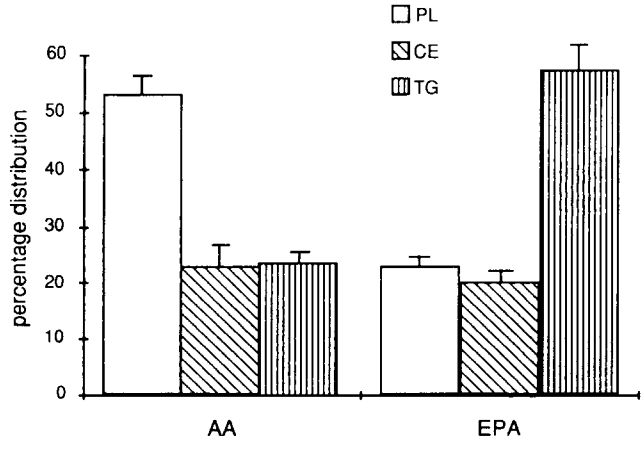

Figure 3 Percentage distribution (average ± SEM) of arachidonic (AA) and eicosapentaenoic (EPA) acids among the major plasma lipid classes in plasma of rabbits fed three different oils.

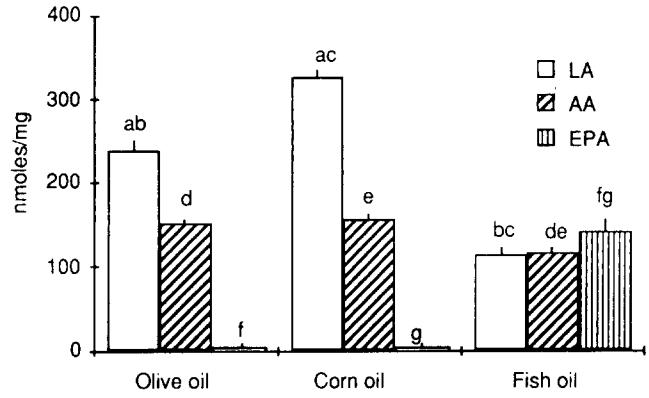

Figure 4 Levels (nmoles/mg total lipid) of linoleic (LA), arachidonic (AA), and eicosapentaenoic (EPA) acids in platelet total lipids. Values (average ± SEM) with the same letter are statistically different from each other at $p < 0.0005$.

group. In this group, EPA was accumulated above AA levels.

The concentrations of LA, AA, and EPA in the four major glycerophospholipids of platelets are shown in Fig. 5. In phosphatidyl ethanolamine (PE) (panel A), levels of LA were elevated in the CO and markedly reduced in the FO group. AA levels were not significantly different in the OO and CO groups and somewhat lower in the FO group. EPA levels in the FO fed animals exceeded those of AA. Group differences of LA levels in phosphatidylcholine (PC) (panel B) followed the same pattern as those in PE. AA levels, in contrast, were not different in the three groups, and, again, in the FO group EPA reached levels higher than those of AA. In phosphatidylserine (PS) (panel C) the trend of differences for LA levels were the same as in PE and PC, but in the FO group AA levels were, unexpectedly, even greater than those of the other groups. In this group, EPA reached the same levels as AA. Levels of AA and EPA in phosphatidyl inositol (PI) (panel D) were one order of magnitude lower than in the other glycerophospholipids. LA levels were negligible in all groups and were not affected by dietary manipulations. AA levels were similar in the OO and CO groups and some reduction, although not significant, occurred in the FO group. EPA was accumulated in the FO group, but levels were quite lower than those of AA. In general, EPA accumulating in the FO group replaced LA rather than AA in platelet PL, with the exception of PI, and most of the reduction of AA occurred in PE. Finally, EPA levels reached or exceeded those of AA in all lipid classes, except for PI.

The patterns of TxB_2 production by platelets stimulated with increasing concentrations of thrombin (Fig. 6) show a linear increase in all groups, with concentrations of the agonist exceeding 2.5 U/ml. No significant difference among groups was, however, observed.

Discussion

The influence of dietary manipulations on the fatty acid patterns of plasma and platelet lipids has been

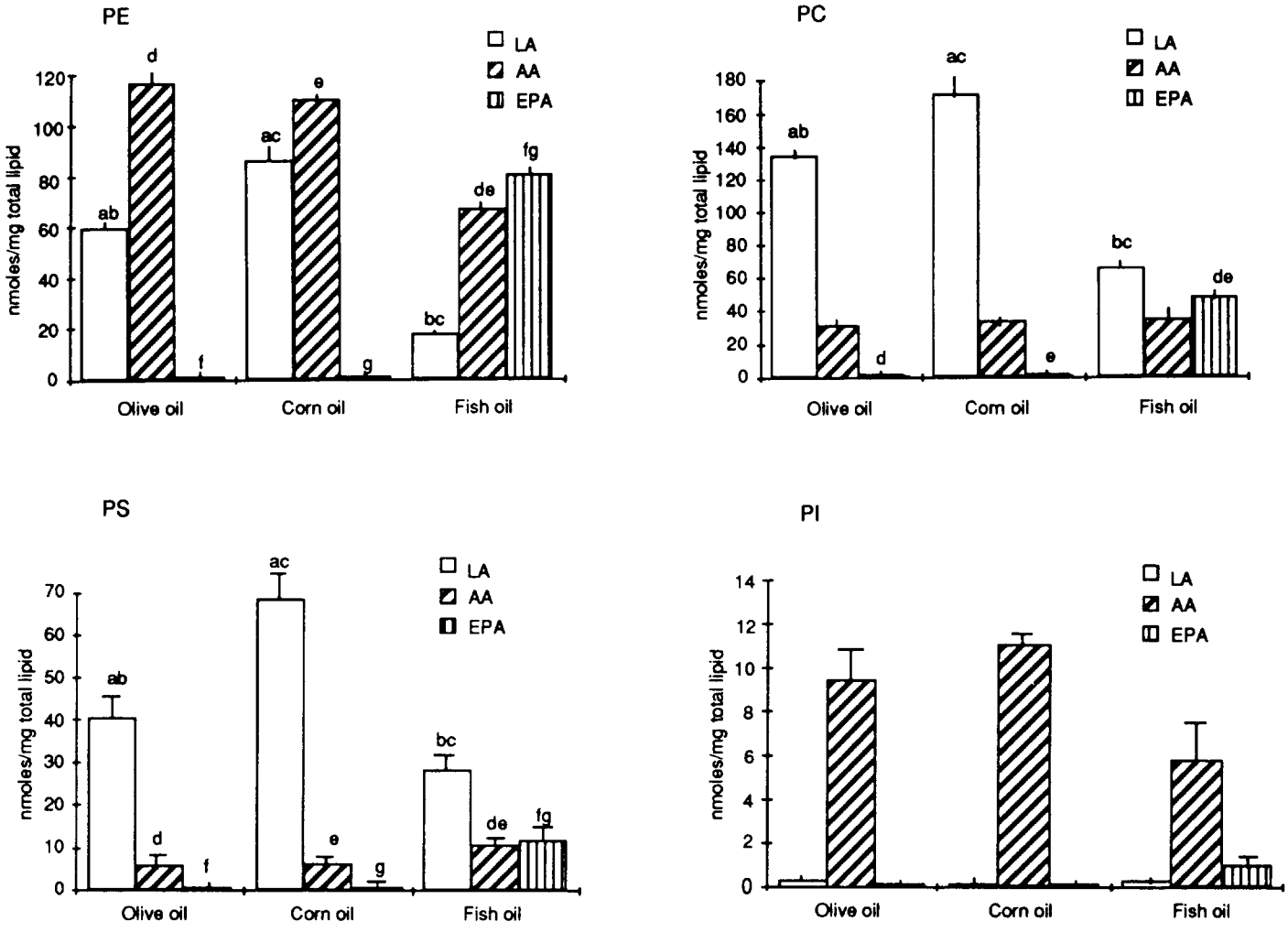

Figure 5 Levels (nmoles/mg total lipid) of linoleic (LA), arachidonic (AA), and eicosapentaenoic (EPA) acids in platelet PE, PC, PS, and PI. Values are the average ± SEM. Values with the same letter are statistically different from each other at the following levels: PE:a,b,c,d,e,f,g:$p < 0.005$. PC:a:$p < 0.01$; b,c,d,e:$p < 0.005$. PS:a,c,d,f,g:$p < 0.0005$; b:$p < 0.025$; e:$p < 0.0025$. PI:a,c,d,f,g:$p < 0.0005$; b:$p < 0.0025$.

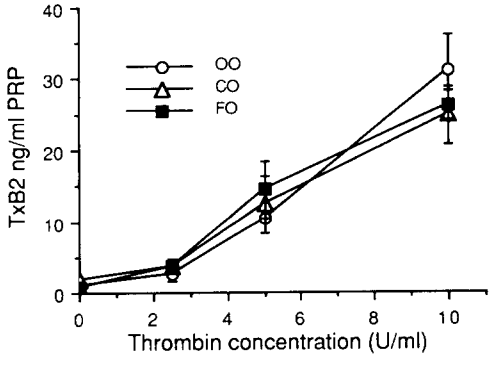

Figure 6 Levels (ng/ml) of TxB_2 in PRP at 2 min after stimulation with increasing concentrations of thrombin.

described in a number of studies,[6,11-15] and, on the basis of the fatty acid compositon of dietary fats, the effects of such manipulations are generally predictable. Here, however, individual fatty acids of the *n*-6 and *n*-3 series were distinctly affected by dietary manipulations. For instance, the administration of either CO or OO, despite inducing remarkable changes of LA levels in plasma lipids, did not appreciably affect total levels of AA as well as the distribution of this PUFA among plasma lipid classes.

In the FO fed animals, levels of all three PUFAs were markedly modified. The expected accumulation of EPA was associated with a reduction of LA, especially in PL and CE, whereas AA levels were surprisingly enhanced, although only slightly. The depletion of LA rather than of AA in the animals fed FO may be the consequence of the relative deficiency in the FO diet. On the other side, the tendency toward a rise of plasma AA, especially in the TG fraction, may well be a consequence of the depletion of tissue AA induced by the administration of *n*-3 fatty acids. The accumulation of EPA in plasma lipids resulted also in

redistribution of AA among the different classes, with a decline in PL and elevation in CE and TG. EPA, which was supplied to the animals as triglyceride, was still mainly present in plasma associated with this fraction, but appreciable amounts could be measured also in PL and CE. These data suggest that EPA of exogenous origin was progressively being transferred from TG to the other lipid classes, and that AA was, at the same time, displaced from PL to CE and TG. Increase of AA content of plasma TG, in addition to elevation of this fatty acid in liver cholesterol esters has also been described in rats fed high levels of *n*-3 fatty acids (EPA 11% and DHA 3% of the energy).[16]

The data on plasma and platelet fatty acids, taken together, suggest that LA stores were depleted in animals fed FO with a relative LA deficiency, as already described by Singer et al.[14] in a human study. It is of interest, however, that the LA fraction associated with TG, which may be the major component of LA transport in plasma, was not reduced, indicating that active transport of LA through plasma TG was still operating after 5 weeks on *n*-6 depletion and suggesting compensatory release of this essential fatty acid from liver into the plasma compartment.

Changes of saturated fatty acids were also observed after administration of *n*-3 fatty acids. The lower incorporation of palmitic acid in plasma TG of the FO fed animals may be related to the reduced TG and VLDL synthesis induced by dietary *n*-3 fatty acids.[12]

Previous studies on the effects of dietary fats on platelet fatty acids were mainly concerned with the changes in the percentage of fatty acid distribution in individual lipid classes, which have been reported to be differentially affected,[15-19] rather than on the changes of pool sizes of specific fatty acids, induced by dietary manipulations. Our study that evaluated the absolute levels of individual fatty acids in platelet lipids (nmoles/mg platelet total lipid) showed that, while differences between the OO and the CO fed animals were small and confined to LA levels, in the FO group differential accumulation of EPA and depletion mainly of LA and, to some extent of AA, occurred among the various lipid classes. The most pronounced changes occurred in PE, since over 50% of EPA accumulation in, and more than 95% of AA loss from, platelets were confined to this lipid class. No reduction of AA occurred in PC and only a small fraction of total AA reduction took place in PI. About 60% of total LA reduction was associated with PC and over 30% with PE. It may be hypothesized that PE acts as a store of cellular AA, in conditions of *n*-6 fatty acid deprivation and *n*-3 supplementation, since the fall of AA in this lipid class was associated with constant levels in other PL. Also, depletion of AA in PE, but not in PC, which, in cell membranes, is mainly located at the outer surface, and elevation of AA in plasma lipids, suggest that different PL are sequentially involved in the outward transport of AA from intracellular lipids into the plasma compartment, under the condition of *n*-3 fatty acid supply combined with *n*-6 deficiency.

Previous studies in the rabbit[20-21] have also shown that the administration of diets containing 40% of the energy as an oil rich in *n*-3 fatty acids did not appreciably modify AA levels in platelet PL. Here, the accumulation of EPA mainly in PE is in agreement with measurements in platelets of human subjects consuming fish oil concentrates, showing accumulation of this fatty acid especially in PE plasmalogen[22] and minimal accumulation in PI.[15] From a quantitative point of view, the greatest changes concerning *n*-6 and *n*-3 PUFAs, in the FO group were observed for LA, which was markedly reduced both in PE and PC. As expected, the reduction of LA in platelet PC, a PL class which is considered to equilibrate with the plasma compartment, followed the decline of this fatty acid in plasma. On the other hand, the reduction in PE may be part of the fatty acid rearrangements compensating for the massive accumulation of EPA.

It is of interest that the formation of thromboxane B_2 by stimulated platelets was not different in the animals fed the three types of oils, despite the appreciable accumulation of EPA in platelet PL, in the animals fed FO. The lack of modification of TxB_2 formation, under these conditions may be due to the lack of changes of AA in PC and PI, phospholipid classes which significantly contribute to the release of this eicosanoid-precursor fatty acid, upon platelet stimulation.[23] Our data on the influence of *n*-3 fatty acids on thromboxane formation are in contrast with results reported in the previously quoted studies,[20,21] in which, however, FO was administered for longer periods and at higher doses.

In conclusion, the administration of a fish oil preparation rich in *n*-3 fatty acids and low in LA to rabbits resulted in selected depletion of LA rather than of AA in plasma and platelet lipids and this was associated with considerable redistribution of *n*-6 fatty acids among lipid classes. The lack of significant changes of AA levels in platelet PC and PI was associated with no modification of TxB_2 synthesis by stimulated platelets. Our data indicate that the typical reduction of 20:4 *n*-6 in plasma and platelet lipids described after administration of *n*-3 fatty acids does not occur if the diet is deficient of LA and this results in no modification of platelet eicosanoid formation. More generally, the data underline the importance of assessing the differential response of lipid pools to the administration of preformed long chain PUFA of the *n*-3 series in the evaluation of their biological effects.

Acknowledgments

This work was supported by contracts CT 87.00422 and CT 87.01424 of CNR.

References

1 Hornstra, G. (1985). Dietary lipids, platelet function, and arterial thrombosis in animals and man. *Proc. Nutr. Soc.* **44**, 371–378

2 Dyerberg, J., Bang, H.O., Stoffersen, E., Moncada, S., and Vane, J.R. (1978). Eicosapentaenoic acid and prevention of thrombosis and atherosclerosis? *Lancet* **ii**, 117–119

3 Galli, C., Agradi, E., Petroni, A., and Tremoli, E. (1981). Differential effects of dietary fatty acids on the accumulation of arachidonic acid and its metabolic conversion through the cyclooxygenase and lipoxygenase in platelets and vascular tissue. *Lipids* **16,** 165–172

4 Tremoli, E., Petroni, A., Socini, A., Maderna, P., Colli, S., Paoletti, R., Galli, C., Ferro-Luzzi, A., Strazzullo, P., Mancini, M., Puska, P., Iacono, J., and Dougherty, R. (1986). Dietary interventions in North Karelia, Finland, and South Italy. Modifications of Thromboxane B_2 formation in platelets of male subjects only. *Atherosclerosis* **59,** 101–111

5 Fischer, S. and Weber, P.C. (1983). Thromboxane A_3 (TxA_3) is formed in human platelets after dietary eicosapentaenoic acid (C 20:5 ω3). *Biochem. Biophys. Res. Commun.* **116,** 1091–1099

6 Siess, W., Roth, P, Kurzmann, L., Boehlig, B., Scherer, B., and Weber, P.C. (1980). Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet* **i,** 441–444

7 Fischer, S. and Weber, P.C. (1984). Prostaglandin I_3 is formed in vivo in man after dietary eicosapentaenoic acid. *Nature (Lond.)* **307,** 165–168

8 Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *Am. Stat. Ass. J.* **50,** 1096–1121

9 Dunnett, C.W. (1964). New tables for multiple comparisons with a control. *Biometrics* **20,** 482–491

10 Granstrom, E., Kindahl, H., Samuelsson, B. (1976). Radioimmunoassay for thromboxane B_2. *FEBS Lett.* **9,** 611–627

11 Herold, P.M. and Kinsella, J.E. (1986). Fish oil consumption and decreased risk of cardiovascular consumption and decreased risk of cardiovascular disease—a comparison of findings from animal and human feeding trials. *Am. J. Clin. Nutr.* **43,** 566–598

12 Harris, W.S., Connor, W.E., Illingworth, R.D., and Foster, D.M. (1984). The mechanism of the hypotriglyceridemic effect of dietary ω3 fatty acids in man. *Clin. Res.* **32,** 560

13 Boberg, M., Vessby, B., and Selinus, I. (1986). Effects of dietary supplementation with *n*-6 and *n*-3 long chain polyunsaturated fatty acids on serum lipoproteins and platelet function in hypertriglyceridemic patients. *Acta. Med. Scand.* **220,** 153–160

14 Singer, P., Jaeger, W., Wirth, M., Voigt, S., and Naumann, E. (1983). Lipid and blood pressure-lowering effect of mackerel diet in man. *Atherosclerosis* **49,** 99–108

15 Galloway, J.H., Cartwright, I.J., Woodcok, B.E., Greave, M., Russell, R.G.G., and Preston, F.E. (1985). Effects of fish oil supplementation on the fatty acid composition of the human platelet membrane: demonstration of selectivity in the incorporation of eicosapentaenoic acid into membrane phospholipid pools. *Clin. Sci.* **68,** 449–454

16 Garg, M.L., Wierzbicki, A., Thomson, A., and Clandinin, M.T. (1989). ω-3 fatty acid increases the arachidonic acid content of liver cholesterol ester and plasma triacylglycerol fractions in the rat. *Biochem. J.* **261,** 11–15

17 Brox, J.H., Killie, J.E., Gunnes, S., and Nordoy, A. (1981). The effect of cod liver oil and corn oil on platelets and vessel wall in man. *Thromb. Haemostas.* **46,** 604–611

18 Schacky, C., Fischer, S., and Weber, P.C. (1985). Long term effects of dietary marine ω3 fatty acids upon plasma and cellular lipids, platelet function and eicosanoid formation in humans. *J. Clin. Invest.* **76,** 1626–1631

19 Mori, T.A., Codde, J.P., Vandongen, R., and Beilin, L.J. (1987). New findings in the fatty acid composition of individual platelet phospholipids in man after dietary fish oil supplementation. *Lipids* **22,** 744–750

20 Hornstra, G., Haddeman, E., Kloeze, J., and Verschiren, P.M. (1980). Dietary-fat-induced changes in the formation of prostanoids of the 2 and 3 series in relation to arterial thrombosis (rat) and atherosclerosis (rabbit). *Adv. Prost. Thromb. Leukotr. Res.* **12,** 193–202

21 Houwelingen, A.C., Hennissen, A.H., Seyberth, H.W., and Hornstra, G.H. (1987). Effect of dietary fat type on Prostaglandin E production in vivo. *Adv. Prost. Thromb. Leukotr. Res.* **17,** 871–879

22 Holub, B.J., Celi, B., and Skeaff, C.M. (1988). The alkenylacyl class of ethanolamine phospholipid represents a major form of eicosapentaenoic acid (EPA)-containing phospholipid in the platelets of human subjects consuming a fish oil concentrate. *Thromb. Res.* **50,** 135–143

23 Broekman, M.J. (1986). Stimulated platelets release equivalent amounts of arachidonate from phosphatidylcholine, phosphatidylethanolamine, and inositides. *J. Lipid Res.* **27,** 884–891