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VITAMIN E INFLUENCES THE EFFECTS OF FISH OIL ON FATTY ACIDS AND EICOSANOID PRODUCTION IN PLASMA AND CIRCULATING CELLS IN THE RAT

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Abstract—An EPA enriched oil (MaxEPA, Seven Seas, U.K. containing 18% EPA and 12% DHA) alone or supplemented with 10 mg/ml/αtocopherol, was administered by gastric intubation at the dose of 3.2 ml/kg/day for a period of eight weeks to male rats fed a standard diet. An additional group of animals was treated with the same amount of olive oil.

The administration of MaxEPA alone resulted, as expected, in accumulation of EPA and reduction of AA levels in plasma, platelet, red blood cell and PMNL phospholipids, when compared to values in the olive oil group. In addition, levels of linoleic acid were elevated, suggesting inhibition of the conversion of linoleic to arachidonic acid. Formation of i.r. TxB_2 by stimulated PRP, of i.r. 6-keto-$PGF_{1\alpha}$ by perfused aortas, and of IR LTB_4 and C_4 by stimulated PMNL were reduced, but production of superoxide anion by PMNL was enhanced by MaxEPA treatment vs the olive oil treatment.

Supplementation of MaxEPA with vitamin E caused a smaller reduction of 20:4 levels and a smaller increase of 20:5 levels in plasma and cell phospholipids and modified the effects of MaxEPA on eicosanoid and superoxide anion production, suggesting that lipid peroxidation may mediate some of the biological effects of ω3 fatty acids.

Several studies in animals and humans have shown that dietary fatty acids modulate eicosanoid production in various types of cells, and these changes are associated, possibly with a causal relationship, with modifications of functional parameters. Particular attention has been devoted to the effects of polyunsaturated fatty acids in the diet, on cells such as platelets, whose function can be studied *ex vivo* and whose activation steps include production of eicosanoids. The administration of 20 carbon polyunsaturated fatty acids (PUFA), potential substrates of the cyclo and lipoxygenase pathways and present at a very low dose in conventional diets, bypass the desaturation and elongation steps which modulate 20 carbon (C) PUFA accumulation in cells and tissues when the predominant PUFAs in the diet are linoleic and alfa linolenic acids.

Administration of eicosapentaenoic acid (20:5 ω3, EPA)—present at a substantial concentration in the diet and in appreciable levels in plasma and tissue lipids [1, 2] of population groups eating large amounts of fish—has been shown in animal and controlled human studies to reduce platelet and leukocyte reactivity [3–9] and to give rise to eicosanoids (thromboxane and leukotrienes) with reduced biological activities, when compared to products of the arachidonic acid cascade. The effects of EPA on functional and biochemical parameters may result, however, from a combined action on various metabolic steps, since 20:5 ω3 may interfere with the metabolism and utilization of ω6 fatty acids, in addition to originating oxygenated products with modified biological actions. Furthermore, differential incorporation of this fatty acid may occur in different plasma lipids and in lipid fractions of circulating cells with different life spans. Finally, since EPA is a highly unsaturated and potentially unstable compound, some of the effects observed after its administration may be related to enhanced lipid peroxidation *in vivo*. In fact, although MaxEPA preparations available for use by humans normally contain 1 mg αtocopherol/ml of oil, this antioxidant level may not be adequate, due to the very high degree of unsaturation of fatty acids present in these oils. We have, thus, comparatively studied the effects of treatment with MaxEPA or with a MaxEPA preparation containing 1% (w/w) alpha-tocopherol. In addition we have evaluated the effects of an oil, such as olive oil, which is relatively low in polyunsaturates and is rich in various antioxidants in addition to tocopherols [10].

The oil preparations were given for a period of eight weeks to male rats fed a standard pellet diet, and thus receiving approximately the same amounts of linoleic acid. The following measurements were carried out: (A) biochemical parameters: fatty acid composition of plasma lipids (phospholipids and cholesterol esters) and of platelet, polymorphonuclear leukocyte (PMNL) and red blood cell (RBC) phospholipids; (B) functional parameters: thromboxane B_2 (TxB_2) formation by stimulated platelet-rich-plasma (PRP) and PMNL; prostacyclin (PGI_2) release from perfused isolated aortic segments; leukotriene (LTB_4 and C_4) release and superoxide anion ($O_2^{\cdot-}$) production by elicited PMNL.

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MATERIALS AND METHODS

Diets. All animals in the study were fed a standard pellet diet. In addition, the following oils were administered, as subsequently described: olive oil, MaxEPA or MaxEPA containing 10 mg/g *dl*-alfa tocopherol (Merck, Darmstadt, F.R.G.). Aliquots of the oil samples corresponding to a single dosage were separately stored at −20°.

The fatty acid compositions of the oils and of the lipids extracted from the standard pellet diet were evaluated by gas liquid chromatography of methyl esters.

Treatments. Three groups of male rats of the Charles River strain (average weight 200 g), kept on a standard pellet diet, were administered by gastric intubation either one of the above oils at the starting dose of 1 ml/animal every other day for a period of 8 weeks, corresponding to about 3.2 ml/kg/day. The dose was increased at the beginning of each week on the basis of body weight increments. The calculated intake of vitamin E through the oil administrations amounted to 25 mg/kg/day for MaxEPA + vitamin E treated rats, 2 mg/kg/day for animals treated with MaxEPA alone and 0.4 mg/kg/day for animals supplemented with olive oil.

Sample preparation. Blood collection was performed by cardiac puncture from anaesthesized animals using sodium citrate (3.13%, 1:9 ml of blood) as anticoagulant.

Plasma was obtained during the preparation of platelets by high speed centrifugation of blood after removal of PRP.

Platelet studies. Platelet-rich-plasma (PRP) was prepared from citrated blood following conventional centrifugation techniques. Platelet number was diluted with homologous platelet-poor-plasma (PPP) to the value of $3 \times 10^5/\mu l$ for determinations of platelet TxB_2 formation. Preparations of washed platelets for studies of the fatty acid composition of phospholipids were obtained by centrifugation of PRP followed by further washing step in EDTA containing buffer [11] in a volume corresponding to 2/5 of the initial plasma volume. Platelets were resuspended, counted and finally subjected to osmotic shock in distilled water and centrifuged at high speed. Pellets were maintained at −80° until analyzed.

Studies on aortic tissue. Aortic tissue was used for the determination of PGI_2 production, evaluated by measuring the levels of 6 keto $PGF_{1\alpha}$ in Tris–HCl buffer which was perfused through isolated aortic segments [12].

Preparation of red blood cell membranes. RBC membranes were prepared from acid citrate dextrose (ACD) anticoagulated blood by centrifugation steps, after PRP removal, followed by further washings in ACD and by a final osmotic shock. A pellet was then obtained by high speed centrifugation and the recovered material was maintained at −80° until analyzed.

Preparation of PMNL. PMNL were separated according to Boyum *et al.* [13]. Briefly, after removal of PRP, the remainder was processed by adding a 1:1 volume of citrate phosphate buffer saline (PBS). PMNL were separated using a standard technique of dextran (Dextran T-500, Pharmacia) sedimentation, centrifugation on Ficoll-Paque (Pharmacia) and hypotonic lysis of red cells. Isolated cells were resuspended at 10^7/ml in PBS containing 0.05% glucose and 0.25% bovine serum albumin (Sigma). All preparations contained more than 97% PMNL. Cell viability was assessed by trypan blue exclusion.

PMNL preparations were used for studies of leukotriene and superoxide anion production after stimulation, and also for analysis of phospholipid fatty acid composition.

Eicosanoid formation. Eicosanoid formation was assessed in stimulated PRP and PMNL as well as in buffers perfused through isolated aortas. Thromboxane formation was evaluated by measuring levels of TxB_2 with a specific RIA [14], in PRP (3×10^5 platelets/μl) at 2 min after stimulation with 5 U NIH/ml thrombin (Topostasin, Roche) and in PMNL (1 ml, 5×10^6 cells, 1 mM Ca^{2+} + 1 mM Mg^{2+}) incubated at 37° (stirring 1000 rpm) for 7 min in the presence of 10 μM calcium ionophore A23187.

Prostacyclin formation in aortic tissue was assessed by measuring 6 keto $PGF_{1\alpha}$ levels in aortic perfusates by a specific RIA [15] and values were expressed as ng/ml/cm^2 of perfused vessel surface.

Levels of LTC_4 and B_4 in PMNL (5×10^6) were measured at 7 min after stimulation with the ionophore A23187 (10 μM), by specific RIAs [16].

Superoxide anion generation was measured in PMNL (3×10^5) cells incubated with 1 mg/ml opsonized zymosan (STZ) for 30 min at 37° using the method of Babior *et al.* [17]. Superoxide anion concentration was calculated as the reduction of ferricytochrome C, which can be inhibited by superoxide dismutase (SOD), using an extinction coefficient of 2.1×10^3 mol l^{-1} cm^{-1} and the results were expressed as nmoles $O_2^-/10^6$ cells/30 min.

Fatty acid analysis of plasma and cell lipids. Lipids were extracted from washed platelets, RBC, PMNL and plasma with a chloroform:methanol 2:1 solvent mixture containing 5 μg/ml of the antioxidant butylated hydroxy toluene (BHT). Total phospholipids were isolated from all lipid extracts, and, in addition, cholesterol esters were isolated from plasma lipids, by thin layer chromatographic techniques using hexane/diethylether/acetic acid (80:20:1) as developing solvents. Methylesters were prepared by acid transmethylation using methanolic HCl (Supelco, Bellefonte, PA) and analyzed by GC on capillary columns (Supelcowax 10, 30 m 0.75 mm i.d., 1.0 μm df) and programmed temperature (135 to 210° at 2.5°/min increments). Analysis of fatty acid methyl esters was performed also on supplemented oils and on lipids extracted from the diet and analyzed. Fatty acid identification was confirmed by the use of reference fatty acid mixtures (Supelco, Bellefonte, PA).

Statistical analysis was carried out by ANOVA.

RESULTS

The fatty acid compositions of the olive and MaxEPA oils and of the lipids extracted from the diet are shown in Table 1. The MaxEPA preparation used in our experiment contained about 16% 20:5 ω3 and 14% 22:6 ω3, whereas the oleic and linoleic

Table 1. Fatty acid percentage composition of oil supplements and of the pellet diet

Fatty acid	MaxEPA	Olive oil	Standard diet
14:0	8.3		
16:0	17.6	17.1	19.8
16:1	8.7		1.5
18:0	2.6		3.3
18:1	14.4	70.0	21.0
18:2	1.7	12.8	52.0
20:0	3.5		—
20:5	15.8		0.7
22:1	5.0		—
22:5	2.0		0.2
22:6	14.0		1.6
U.I.	205	96	141

U.I. = unsaturation index (sum of percentages of individual fatty acids × number of double bonds).

acid contents of olive oil were 70% and 13% respectively. The average daily intakes of total 20 and 22 C ω3 PUFA through the administration of MaxEPA was about 600 mg/kg. Lipids extracted from the standard pellet diet contained linoleic as the major fatty acid, but appreciable concentrations of ω3 fatty acids, especially 22:6, were also present. The unsaturation index of MaxEPA preparation was double that of olive oil, which was even lower than that of lipids extracted from the diet. The calculated daily intakes as energy percentage of total fat and of the major unsaturated fatty acids in the three groups of animals are reported in Table 2. The intakes of total fat and of linoleic acid were substantially the same in all groups of animals, but the intakes of a few individual unsaturated fatty acids were different. An appreciable intake of ω3 fatty acids, especially 22:6, was supplied by the standard diet.

The fatty composition of plasma phospholipids (PL) and cholesterol esters (CE) and of PL in platelets, RBC and PMNL are shown in Tables 3–6. The administrations of MaxEPA, or MaxEPA + Vitamin E or olive oil resulted in different fatty acid compositions of the lipid fractions and changes followed the same trend in plasma and cellular lipids in each treatment group.

When plasma lipids are considered (Table 3), it

Table 2. Calculated daily intakes (as % of energy) of total fat and unsaturated fatty acids

	(a) Through supplements			Total (a + b)	
	MaxEPA and MaxEPA + E	Olive oil	(b) Through diet	MaxEPA and MaxEPA + E	Olive oil
Total fat	4.6	4.6	9.0	13.6	13.6
18:1	0.67	4.0	1.9	2.6	5.9
18:2	0.08	0.7	4.6	4.7	5.3
20:5	0.73	—	0.06	0.79	0.06
22:6	0.65	—	0.14	0.79	0.14

Table 3. Fatty acids of plasma lipids

	Phospholipids			Cholesterol esters		
Fatty acids	MaxEPA (6)	MaxEPA + E (6)	Olive (6)	MaxEPA (6)	MaxEPA + E (6)	Olive (6)
16:0	28.6 ± 0.4	26.6 ± 2.0	27.5 ± 0.7	9.4 ± 0.5^{a}	9.3 ± 0.3^{b}	7.4 ± 0.8^{ab}
18:0	22.7 ± 0.8	21.5 ± 0.6	22.7 ± 0.9	0.7 ± 0.1	0.8 ± 0.03	0.6 ± 0.1
18:1	7.3 ± 0.6	6.6 ± 0.1	7.9 ± 0.9	8.2 ± 0.6^{a}	7.7 ± 0.5^{b}	9.8 ± 1.3^{ab}
18:2	25.6 ± 0.5^{ab}	28.7 ± 0.9^{bc}	22.8 ± 0.5^{ac}	33.5 ± 1.9^{a}	33.5 ± 2.3^{b}	25.2 ± 0.7^{ab}
20:3 ω6	1.0 ± 0.08	0.8 ± 0.1	0.7 ± 0.1^{a}	—	—	—
20:4 ω6	9.4 ± 0.4^{ab}	10.7 ± 0.5^{bc}	14.4 ± 0.3^{ac}	34.1 ± 1.5^{a}	37.0 ± 2.0^{b}	50.1 ± 0.8^{ab}
20:5 ω3	1.2 ± 0.27^{a}	1.0 ± 0.06	0.5 ± 0.06^{a}	4.6 ± 0.7^{a}	2.7 ± 0.4^{b}	0.5 ± 0.1^{ab}
22:5 ω3	1.0 ± 0.22	1.4 ± 0.24	1.2 ± 0.1	1.8 ± 0.3	2.0 ± 0.5	0.7 ± 0.1
22:6 ω3	3.0 ± 0.63	3.0 ± 1.3	2.0 ± 0.5	7.6 ± 0.8^{a}	7.0 ± 0.2b	5.3 ± 0.3^{ab}
Sat	51.3 ± 1.0	48.1 ± 1.7	50.2 ± 0.8	10.1 ± 0.5^{a}	10.1 ± 0.4^{b}	8 ± 0.3^{ab}
Mono	7.3 ± 0.7	6.6 ± 1.1	7.9 ± 0.9	8.2 ± 0.7	7.7 ± 0.6	9.8 ± 1.4
Poly	41.3 ± 0.5^{a}	45.5 ± 1.7^{a}	41.9 ± 0.6	81.6 ± 1.1	82.2 ± 1.0	82.2 ± 0.9
ω6	36.0 ± 0.5^{a}	40.2 ± 0.7^{ab}	37.9 ± 0.4b	67.6 ± 1.7^{a}	70.5 ± 2.3^{b}	77.0 ± 0.9^{ab}
ω3	5.2 ± 0.5^{a}	5.4 ± 0.6^{b}	3.7 ± 0.3^{ab}	14.0 ± 0.7^{a}	11.7 ± 0.4^{a}	6.5 ± 0.3^{a}
ω3/ω6	0.14	0.13	0.10	0.21	0.16	0.08
U.I.	128 ± 3	139 ± 5	135 ± 1	289 ± 10	288 ± 10	298 ± 3
20:4/18:2	0.37 ± 0.04^{a}	0.36 ± 0.03^{b}	0.60 ± 0.02^{ab}	1.00 ± 0.07^{a}	1.10 ± 0.14^{b}	2.2 ± 0.30^{a}
20:5/20:4	0.13 ± 0.04^{ab}	0.09 ± 0.006^{bc}	0.03 ± 0.009^{ac}	0.39 ± 0.11^{ab}	0.07 ± 0.04^{bc}	0.01^{ac}

Values are expressed as weight percentages of total fatty acids and are the means ± SEM. Numbers in brackets indicate the number of samples analyzed; sat: saturated fatty acids; mono: mono unsaturated fatty acids; poly: polyunsaturated fatty acids; U.I.: unsaturation index (sum of percentages of individual fatty acids × number of double bonds).

Values in the same line sharing the same superscript are significantly different from each other.

Table 4. Fatty acids of platelet phospholipids

Fatty acids	MaxEPA (6)	MaxEPA + E (6)	Olive (6)
16:0	35.8 ± 0.5[a]	34.1 ± 0.3[b]	38.1 ± 1.2[ab]
18:0	17.6 ± 0.3[a]	18.9 ± 0.2[ab]	17.1 ± 0.7[b]
18:1	5.4 ± 0.3[a]	5.6 ± 0.3[b]	7.0 ± 0.3[ab]
18:2	11.3 ± 0.3[a]	10.5 ± 0.2[b]	8.0 ± 0.4[ab]
20:3 ω6	0.6 ± 0.3[a]	0.6 ± 0.06[b]	0.4 ± 0.06[ab]
20:4 ω6	19.8 ± 0.3[a]	20.9 ± 0.3[a]	25.3 ± 1.2[a]
20:5 ω3	4.4 ± 0.2[a]	3.9 ± 0.2[b]	1.0 ± 0.1[ab]
22:5 ω3	2.7 ± 0.1	2.8 ± 0.1	1.1 ± 0.3
22:6 ω3	2.6 ± 0.04	2.6 ± 0.2	1.9 ± 0.3
Saturates	53.4 ± 0.7	53.0 ± 0.7	55.2 ± 1.3
Monounsaturates	5.4 ± 0.2[a]	5.6 ± 0.3[b]	7.0 ± 0.3[ab]
Polyunsaturates	41.2 ± 1.0[a]	41.3 ± 0.9[b]	37.7 ± 0.6[ab]
ω6	31.7 ± 0.7	32.0 ± 0.6	33.7 ± 0.4
ω3	9.4 ± 0.3	9.3 ± 0.1	4.0 ± 0.2
ω3/ω6	0.30	0.29	0.12
U.I.	159 ± 5	161 ± 3	147 ± 7
20:4/18:2	1.68 ± 0.11[a]	1.97 ± 0.07[a]	3.23 ± 0.32[a]
20:5/20:4	0.21 ± 0.006[a]	0.19 ± 0.001[b]	0.04 ± 0.004[ab]

Values are expressed as weight percentages of total fatty acids and are the means ± SEM. Numbers in brackets indicate the number of samples analyzed. U.I. = unsaturation index (sum of percentages of individual fatty acids × number of double bonds).

Values in the same line sharing the same superscript are significantly different from each other.

appears that the administration of MaxEPA vs that of olive oil induced elevation of 20:5 ω3 especially in CE. Levels of 22:6 ω3 were also higher, but the difference was significant only in CE. Levels of 18:1 were higher in CE of the olive oil group. Concomitantly, levels of linoleic acid were elevated and those of 20:4 were reduced. These differences were very remarkable in cholesterol esters.

The 20:4/18:2 ratio, which provides a rough index of the conversion of 18:2 to 20:4 was about 50% lower in the MaxEPA treated rats in respect to values in the olive oil group. The 20:5/20:4 ratio was instead about 5-fold higher in the animals fed fish oil. In spite of the significant differences in the levels of linoleic acid (LA), arachidonic acid (AA) and EPA between the MaxEPA and the olive oil treated

Table 5. Fatty acids of red blood cell phospholipids

Fatty acids	MaxEPA (6)	MaxEPA + E (6)	Olive (6)
16:0	33.3 ± 0.5	32.0 ± 1.2	32.3 ± 2.0
18:0	17.4 ± 0.2	17.5 ± 0.5	17.1 ± 0.4
18:1	9.0 ± 0.3[a]	9.5 ± 0.4[b]	11.0 ± 0.3[ab]
18:2	11.9 ± 0.3[a]	11.8 ± 0.2[b]	10.5 ± 0.5[ab]
20:4 ω6	18.4 ± 0.3[a]	19.6 ± 0.2[a]	22.5 ± 1.2[a]
20:5 ω3	1.9 ± 0.05[a]	1.8 ± 0.1[b]	1.0 ± 0.2[ab]
22:5 ω3	3.9 ± 0.1[a]	3.7 ± 0.1[b]	2.5 ± 0.3[ab]
22:6 ω3	4.1 ± 0.1[a]	4.0 ± 0.2[b]	2.6 ± 0.2[ab]
Saturates	50.7 ± 0.5[a]	49.5 ± 0.7[b]	49.4 ± 1.8[ab]
Monounsaturates	9.0 ± 0.3[a]	9.5 ± 0.5[b]	11.0 ± 0.3[ab]
Polyunsaturates	40.2 ± 0.7[a]	40.9 ± 0.6	39.4 ± 1.4[a]
ω6	30.3 ± 0.3	31.4 ± 0.2	33.0 ± 0.8
ω3	9.9 ± 0.2	9.5 ± 0.1	6.1 ± 0.3
ω3/ω6	0.33	0.30	0.18
U.I.	160 ± 3	163 ± 3	157 ± 7
20:4/18:2	1.55 ± 0.51[a]	1.66 ± 0.03[b]	2.14 ± 0.03[ab]
20:5/20:4	0.10 ± 0.03[a]	0.09 ± 0.03[b]	0.05 ± 0.01[ab]

Values are expressed as weight percentages of total fatty acids and are the means ± SEM. Numbers in brackets indicate the number of samples analyzed. U.I. = unsaturation index (sum of percentages of individual fatty acids × number of double bonds).

Values in the same line sharing the same superscript are significantly different from each other.

Table 6. Fatty acids of PMNL phospholipids

Fatty acids	MaxEPA (3)	MaxEPA + E (3)	Olive (3)
16:0	29.1 ± 1.3	30.5 ± 4.0	35.7 ± 2.3
18:0	22.8 ± 0.3[a]	17.7 ± 0.3[a]	15.1 ± 0.7[a]
18:1	15.2 ± 2.0	13.2 ± 0.4	15.4 ± 0.9
18:2	10.0 ± 1.0[a]	10.1 ± 1.0[b]	7.9 ± 0.9[ab]
20:3 ω6	0.7 ± 0.03	0.7 ± 0.06	0.8 ± 0.07
20:4 ω6	11.2 ± 1.2[ab]	17.4 ± 1.6[a]	20.8 ± 1.7[b]
20:5 ω3	2.0 ± 0.2[a]	1.7 ± 0.2[b]	0.4 ± 0.05[ab]
22:5 ω3	2.8 ± 0.3	2.3 ± 0.2	0.7 ± 0.3
22:6 ω3	6.2 ± 0.8	6.4 ± 1.1	3.3 ± 0.3
Saturates	51.9 ± 1.1	48.2 ± 3.1	50.8 ± 2.3
Monounsaturates	15.2 ± 1.8	13.2 ± 0.4	15.4 ± 0.9
Polyunsaturates	32.9 ± 1.9[ab]	38.6 ± 2.6[a]	33.9 ± 2.8[b]
ω6	21.9 ± 1.1	28.2 ± 1.2	29.5 ± 1.2
ω3	11.0 ± 0.5	10.4 ± 0.5	4.4 ± 0.4
ω3/ω6	0.50	0.37	0.15
U.I.	143 ± 2[ab]	163 ± 4[a]	142 ± 3[b]
20:4/18:2	1.12 ± 0.10[a]	1.73 ± 0.60[a]	2.63 ± 0.70[a]
20:5/20:4	0.18 ± 0.01	0.10 ± 0.01	0.02 ± 0.001

Values are expressed as weight percentages of total fatty acids and are the means ± SEM. Numbers in brackets indicate the number of samples analyzed. U.I. = unsaturation index (sum of percentages of individual fatty acids × number of double bonds).

Values in the same line sharing the same superscript are significantly different from each other.

groups, the levels of total PUFA, the total levels of unsaturation (unsaturation index) and the classes of unsaturation were not remarkably different. However, the proportions of ω3 fatty acids in respect to total PUFA were significantly higher in lipids of MaxEPA and of MaxEPA + E treated rats.

The supplementation of MaxEPA with vitamin E, resulted in significant, although quantitatively modest, elevations of PUFA of the ω6 series in both PL and CE and in reduction of PUFA of the ω3 series in CE, with respect to values in the MaxEPA group. The 20:4/18:2 ratio, however, remained unchanged. Levels of 20:5/20:4 ratios were significantly lower in plasma lipids of the MaxEPA + E group in respect of those of the MaxEPA group.

In platelet phospholipids (Table 4), differences between the MaxEPA and olive oil groups were similar to those observed in plasma lipids: higher 18:1 levels in the olive oil group and higher 18:2 and lower 20:4 in the MaxEPA groups. The 20:4/18:2 ratios were 50% lower in the MaxEPA group, and the 20:5/20:4 ratio 5-fold higher, as result of the elevation of 20:5 in the MaxEPA group. Levels of 22:5 and 22:6 were appreciable in the MaxEPA treated group and they were detected also in the olive oil group, in which, however, 22:6 rather than 20:5 was the major ω3 fatty acid.

Levels of PUFA were somewhat higher in the MaxEPA group in respect to the olive oil group in relation to the much greater levels of ω3 fatty acids. The unsaturation index and the unsaturation levels were, instead, similar. In the MaxEPA + E group, levels of 18:0, of 20:4 and of the 20:4/18:2 ratio were significantly higher than in the MaxEPA group. Levels of 20:5, however, were comparable to those in the MaxEPA group.

In RBC phospholipids (Table 5) differences in fatty acid distribution among groups were qualitatively similar, although less pronounced than in platelets. 18:1, 18:2 and 20:5 were elevated and 20:4 was reduced in MaxEPA vs olive. In the MaxEPA group levels of both 22:5 and 22:6 were even greater than those of 20:5. Again in the MaxEPA + E group, levels of 20:4 but not those of 20:5, were elevated over those in the MaxEPA alone group.

In PMNL phospholipids (Table 6), levels of 18:0, 18:2, 20:4 and 20:5 and the 20:4/18:2 and 20:5/20:4 ratios were significantly different in the MaxEPA vs the olive oil group as observed in platelets and RBC.

The difference in the 20:4 levels were, however, much greater than in the other types of cells. In the MaxEPA + E group, values of 20:4 were much higher than in the MaxEPA group, and close to those of the olive oil group, whereas those of 20:5 were the same in MaxEPA and MaxEPA + E.

The administration of MaxEPA, as expected, affected the production of various eicosanoids in stimulated platelets and PMNL, as well as eicosanoid release from vessel walls. Thromboxane formation by stimulated PRP, measured as accumulation of immunoreactive (i.r.) TxB_2, as well as the release of i.r. 6 keto $PGF_{1\alpha}$ from perfused aortas (Fig. 1) were significantly reduced in respect of the values found in the olive oil group. Values for TxB_2 and 6 keto $PGF_{1\alpha}$ formation in the group treated with MaxEPA + E were intermediate between those in the other two groups, although not statistically different from them.

Values for i.r. LTB_4 and i.r. TxB_2 production by PMNL stimulated with the calcium ionophore A

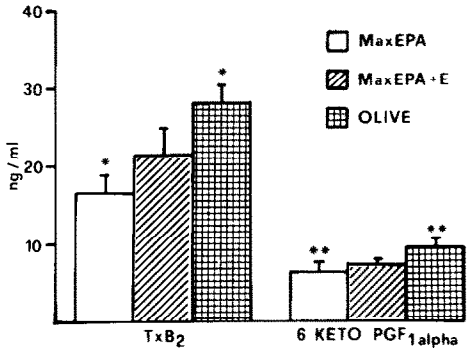

Fig. 1. Levels of i.r. TxB_2 (ng/ml) in PRP (3×10^5 platelets/μl) at 2 min after stimulation with 5 U NIH Thrombin and of i.r. 6-keto-PGF_1 (ng/ml/cm^2 of perfused vessel surface) in aortic perfusates. * Significantly different from each other ($P < 0.02$). ** Significantly different from each other ($P < 0.05$).

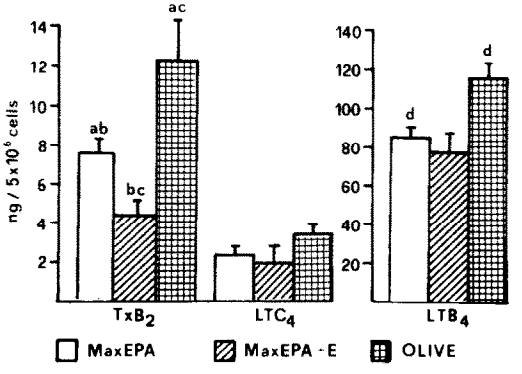

Fig. 2. Levels (ng/5×10^5 cells) of i.r. TxB_2, LTB_4 and LTC_4 in PMNL preparations at 7 min after stimulation with 10 μM A23187. Values sharing the same superscript are significantly different from each other at the following levels: (a) $P < 0.05$, (b) $P < 0.02$; (c) $P < 0.01$, (ed) $P < 0.005$.

23187 were also reduced in the MaxEPA vs the olive oil group, whereas the difference for LTC_4 values was not statistically significant (Fig. 2). Supplementation of MaxEPA with vitamin E tended to reduce eicosanoid production further, this potentiating effect being statistically significant for TxB_2.

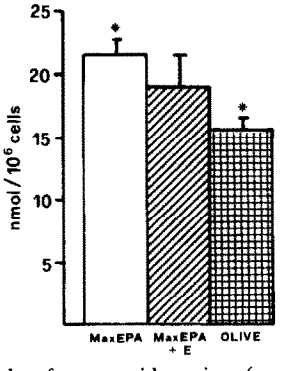

Fig. 3. Levels of superoxide anion (nmol/10^6 cells) in PMNL preparations at 30 min after incubation with 1 mg/ml opsonized zymosan. * Significantly different from each other ($P < 0.05$).

In contrast with the effects of MaxEPA on PMNL eicosanoids, superoxide anion production in MaxEPA treated rats was greater than that in the olive oil group (Fig. 3), and, again, in the MaxEPA + E group, values were somewhat reduced in respect of those in the MaxEPA treated rats.

DISCUSSION

Administration of a lipid fraction enriched with ω3 fatty acids resulted, as already shown by several investigators (for a review see Ref. 18), in significant changes of the fatty acid composition of plasma and cellular lipids, as well as of the production of various eicosanoids by platelets, PMNL and cells of the aortic walls. The major effects appeared to be the incorporation of EPA in cellular lipids and the consequent changes in eicosanoid production. However, interactions between ω6 fatty acids in the diet and in tissues with the administered ω3 fatty acids, as well as changes in lipid peroxidation processes, possibly induced in tissues by the administration of the highly unsaturated ω3 fatty acids, may play a role in the overall effects of EPA. One important aspect in the observed effects of EPA administration was an obvious interaction with the conversion of 18:2 to 20:4 and with the incorporation of the product in cellular lipids. In fact, levels of 18:2, which was supplied in almost equal amounts through the diet of all animal groups, were increased in all lipid fractions analyzed in the groups supplemented with ω3 fatty acids and, in contrast, levels of 20:4 were considerably reduced when compared to the values in the olive oil group. It should be noted, however, that this increment of 18:2 levels in phospholipids has not been observed in human studies. Differences in 18:2 intake in subjects treated with MaxEPA or differences in ω6 fatty acid metabolism between the two species may explain this discrepancy. Reduction of 20:4 levels were substantial in PMNL phospholipids in the MaxEPA group. The 20:4/18:2 ratios were, as a consequence, significantly reduced, the maximal reduction being observed in PMNL and platelets. This effect appeared to be related to the accumulation of 20:5, expressed also as an increment of the 20:5/20:4 ratios, which was more pronounced in platelets and PMNL than in other cells. Remarkable accumulation of 20:5 and especially 22:6 occurred in plasma CE, suggesting that EPA was very efficiently transferred from phospholipids to cholesterol through the LCAT activity. DHA was present in appreciable concentrations also in CE from the olive oil treated group, suggesting that 22:6 steadily supplied through the intake of the standard diet was efficiently incorporated in CE.

The effects of vitamin E supplementation on levels of ω6 and ω3 fatty acids in plasma and cellular lipids were to some extent unexpected, since 20:4 levels were elevated, especially in PMNL, when compared to the values in the MaxEPA alone, but the antioxidant did not influence the levels of 20:5. In plasma CE, levels of 20:5 were even reduced in the MaXEPA + E vs the MaxEPA alone group. These observations suggest that the 20:4 lowering effect

induced in cellular lipids by dietary ω3 fatty acids was mediated, to some extent, by favouring peroxidation processes, which could be affected by vitamin E supplementation. The mechanism(s) responsible for the lower reduction of 20:4 observed in phospholipids of animals treated with MaxEPA plus vitamin E, in respect of values found in samples of the MaxEPA treated group, appear(s) to be rather complex. However, since the 20:4/18:2 ratio was not significantly different in the two groups, an influence on the LA to AA conversion is very unlikely. Direct effects of vitamin E on 20:4 utilization, e.g. for eicosanoid production, may be responsible for the above modifications of 20:4 levels.

The effects of EPA on platelet thromboxane and arterial prostacyclin production were in accordance with previous observations [18]. It should be noted, however, that in our study the production of Δ17-6 keto $PGF_{1\alpha}$ was not evaluated. The somewhat higher eicosanoid productions by platelets and cells of the arterial wall in the group treated with MaxEPA + E, in respect to those in the MaxEPA alone group might reflect the retention of higher 20:4 levels in the precursor pools.

The effects of EPA on eicosanoid formation in PMNL were similar to those observed in platelets. More specifically, the production of TxB_2 by PMNL, although relatively low in comparison with that of platelets [19], and that of LTB_4 were reduced by EPA. However, the influence of ω3 fatty acids on PMNL was more complex, since superoxide anion formation, in contrast with the effects on eicosanoids, was enhanced in PMNL from the MaxEPA group, suggesting that this process might have been favoured by the administration of highly unsaturated and, thus, potentially highly oxidizable, compounds.

The lowering effects of vitamin E supplementation on both eicosanoid and superoxide anion production, in respect to values in the MaxEPA group suggests that generation of lipid peroxides plays a role in the overall process of PMNL activation. In fact, eicosanoid production in the MaxEPA + E group was lower than in the MaxEPA group, in spite of the considerably higher levels of 20:4 in the former. This suggests that vitamin E supplementation potentiated the lowering effects on eicosanoid production in PMNL exerted by EPA in respect of olive oil.

In conclusion, our results, while confirming the inhibiting activities of EPA on cellular eicosanoid formation, indicate that complex interactions occur between exogenous ω3 fatty acids and metabolism of ω6 fatty acids, and suggests that additional mechanisms in the activity of ω3 fatty acids on cellular activation processes and on the 20:4 cascade may be mediated by processes such as lipid peroxidation, which are affected by antioxidants.

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