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INCREASED GLUTATHIONE PEROXIDASE ACTIVITY IN HUMAN BLOOD MONONUCLEAR CELLS UPON *IN VITRO* INCUBATION WITH N-3 FATTY ACIDS

CATHERINE JOULAIN, ANNIE F. PRIGENT,* GEORGES NÉMOZ and MICHEL LAGARDE

Institut National de la Santé et de la Recherche Médicale U 352, Laboratoire de Chimie Biologique, Institut National des Sciences Appliquées de Lyon, 20 avenue Albert Einstein, 69621 Villeurbanne Cédex, France

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Abstract—Fish oil-enriched diets have been shown to increase the n-3 polyunsaturated fatty acid (PUFA) content of cell membranes, *in vivo*, and to simultaneously enhance the glutathione peroxidase (glutathione: H_2O_2 oxidoreductase, EC 1.11.1.9) (GSH-Px) activity of platelets and erythrocytes both in animals and humans. The present study aimed to determine whether *in vitro* enrichment of human peripheral blood mononuclear cells (PBMC) with either eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) might have similar influence on the GSH-Px activity of these cells. Pretreatment of human PBMC for a short period of time (90 min) with 5 μM of either EPA or DHA was sufficient to induce a significant enrichment of cellular phospholipids in the corresponding fatty acid. This was accompanied by an increased GSH-Px activity of these cells. This stimulatory effect proved to be specific of n-3 fatty acids since it was not observed with saturated, monounsaturated or n-6 polyunsaturated fatty acids. Neither EPA nor DHA had a direct influence on the GSH-Px activity of PBMC in cell-free preparations. Both fatty acids had no influence on the distribution of total proteins between the cytosolic and the particulate compartments. EPA did not significantly alter the GSH-Px kinetic behavior thus suggesting an increased expression of the enzyme. In addition, DHA slightly but significantly decreased the proliferative response of PBMC to the mitogenic stimulation by Con A whereas EPA only tended to do so.

Key words: Con A; DHA; EPA; glutathione peroxidase; human mononuclear cells

Animal experiments and human trials have promoted fish oil and n-3 fatty acid concentrates from fish oil for the prevention of cardiovascular and inflammatory diseases [1]. However, excessive intake of fat is associated with impaired immune responses including a decreased capacity of immune cells to recognize foreign stimuli, and a lower proliferative response of T-cells to mitogens [2, 3]. GSH-Px† (EC 1.11.1.9) is a key enzyme involved in the reduction of various hydroperoxides at the expense of GSH [4]. Besides hydrogen peroxide, GSH-Px is also able to reduce a large variety of organic hydroperoxides including lipid hydroperoxides derived from arachidonic acid. Several clinical trials have shown that oral supplementation with fish oil-enriched diet increased GSH-Px activity both in erythrocytes and platelets [5–7]. In psoriatic patients, plasma membranes of skin cells and erythrocytes have been shown to contain higher levels of PUFAs especially arachidonic acid, than cells from healthy subjects [8]. Interestingly, GSH-Px activity in both erythrocytes and platelets from psoriatic patients was significantly increased when compared with normal cells [8]. Moreover, fish oil supplementation, which further increased the PUFA content of the cells, also induced a further stimulation of GSH-Px activity in psoriatic subjects [8]. It has been assumed that the enrichment of membrane phospholipids with n-3 PUFAs following the administration of fish oil-enriched diet might generate lipid hydroperoxides able to directly increase GSH-Px activity [7]. Similar GSH-Px activation has been described in erythrocytes after *in vitro* incubation with acetylphenylhydrazine known to release superoxides from haemoglobin [9].

The aim of the present study was to examine the influence of EPA (20:5 n-3) and DHA (22:6 n-3) the two main fatty acids of fish oil, on the GSH-Px activity of human PBMC following a short incubation period (90 min) of the cells with the fatty acids complexed to the albumin of the culture medium. An eventual link between the alteration of GSH-Px activity and an effect of these fatty acids on the lymphoproliferative response was also investigated.

MATERIALS AND METHODS

Chemicals. [Methyl-^{3}H]thymidine (50 Ci/mmol) was obtained from Dositek (Orsay, France). [5, 6, 8, 9, 11, 12, 14, 15 – ^{3}H]arachidonic acid (200 Ci/mmol) was from Amersham International (Les Ullis, France). Lymphocyte preparation medium, RPMI

* Corresponding author: A. F. Prigent, Chimie Biologique, Bâtiment 406, Institut National des Sciences Appliquées de Lyon, 69621 Villeurbanne cédex, France. Tel. (33) 72.43.85.71; FAX (33) 72.43.85.24.

† Abbreviations: ConA, concanavalin A; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GSH, glutathione; GSH-Px, glutathione peroxidase; PBMC, peripheral blood mononuclear cells; PUFA, polyunsaturated fatty acid.

1640 (with HEPES), human AB serum, glutamine, streptomycin, penicillin were purchased from J Bio (Paris, France). FAs (16:0, 18:0, 18:1n − 9, 18:2n − 6, 20:5n − 3, 22:6n − 3), Con A, β-NADPH tetrasodium salt, *t*-butyl hydroperoxide, GSH reductase (Type III) were obtained from Sigma-Chimie (L'Isle d'Abeau, France). GSH (reduced) was from Boehringer Mannheim (Meylan, France).

Preparation of human PBMC. Venous blood from healthy subjects who had not taken any medication for 2 weeks prior to blood donation was drawn into citric acid–sodium phosphate–dextrose anticoagulant. PBMC were separated by dextran sedimentation and density gradient centrifugation through lymphocyte preparation medium (J Bio, Paris, France), washed with saline phosphate buffer and then washed twice with RPMI 1640 by low speed centrifugations in order to eliminate more thoroughly the contaminating platelets. Cell viability checked with Trypan blue staining was routinely higher than 95%.

FA preparations. Free FAs were stored at −20° in ethanol under nitrogen. Aliquots of the ethanolic solution were evaporated to dryness under reduced pressure. RPMI 1640 medium containing 1% human AB serum was added to give a final FA concentration of 5 μM and an albumin to FA ratio of 1. The mixtures were incubated at 37° overnight.

Experimental incubations. PBMC were incubated for 90 min at 37° in RPMI medium containing the FAs complexed to serum albumin. Control cells were incubated in the same conditions without FAs. At the end of the incubation period, cells were pelleted and washed three times with the Phillips' buffer and disrupted according to Caldwell *et al.* [10] as described previously [11]. At the end of the lysis procedure, homogenates were centrifuged for 1 hr at 105,000 *g*. Supernatant fractions were stored at −80° until GSH-Px assays.

In some experiments, 5 μg of Con A per 10^6 cells were added to cells preincubated with EPA or DHA for 60 min, or to control cells preincubated with albumin alone. Cells were incubated further for 30 min at 37°. Unstimulated cells were incubated for the same length of time without Con A. At the end of the incubation period, cells were treated as described above.

GSH-Px assay. GSH-Px activity was determined according to the method of Paglia and Valentine [12] as modified by Chaudière and Gérard [13]. The assay mixture consisted of 50 mM Tris–HCl buffer, pH 7.6, 1 mM EDTA, 1 mM GSH, 0.125 mM NADPH, H^+ and 1 U/mL GSH reductase. Cell supernatants were preincubated for 2 min at 37° in the assay mixture and the reaction was initiated by the addition of 0.366 mM *t*-butylhydroperoxide. Absorbance at 340 nm was recorded in a Beckman DU8 spectrophotometer. Results are expressed in nmol NADPH oxidized per min per mg protein using an extinction molar coefficient of 6.22/cm²/μmol for NADPH. Proteins were assayed according to Bradford using bovine serum albumin as a standard [14]. Inhibition (95%) was consistently observed in the presence of 0.2 mM mercaptosuccinic acid which indicates a selenium dependent GSH-Px activity. In kinetic experiments, *t*-butylhydroperoxide concentrations varied from 0.1 to 0.8 mM. For each *t*-butylhydroperoxide concentration, GSH varied from 0.5 to 6 mM. Kinetic data were analysed using a computerized non-linear curve fitting program AJNL for Macintosh INRA/INSA.

FA composition of phospholipids. In experiments designed to control the FA composition of cell phospholipids, PBMC were labeled with 1 nM [^{3}H]-arachidonic acid for 1 hr at 37° as described previously [15] before the incubation in the presence of the FAs. Lipids were extracted as in Ref. 15 and lipid samples were chromatographed on TLC plates developed with hexane/diethyl ether/acetic acid (70/30/1 by vol.) which left phospholipids at the origin. Phospholipids were scraped off from the plate and directly transmethylated with 14% boron trifluoride in methanol, at 100° for 90 min under N_2 atmosphere, according to Morrisson and Smith [16]. Fatty acid methyl esters were quantified by gas–liquid chromatography using a 0.25 mm × 60 m SP 2380 capillary column (Supelco, Bellefonte, PA, U.S.A.) and helium as a carrier gas, as described previously [17]. The oven temperature was programmed from 145° to 210° at 0.9°/min. FAs were identified by retention time comparisons with standard FA methyl esters [18]. In control experiments performed on the same sample of human plasma to check the reliability of the FA analysis, SDs were always found to be lower than 10% whatever the considered FA.

Cell cultures. Cells were cultured in wells of microtitre culture plates (approx. 2×10^5 cells/well) in RPMI + human AB serum previously incubated overnight at 37° with or without FAs. Culture medium was supplemented with 2 mM glutamine, 100 μg of streptomycin/mL and 100 U of penicillin/mL. Con A (5 μg/mL) was added at the initiation of the culture. Cultures were incubated at 37° in an air–CO_2 (95/5) atmosphere. After incubation for 48 hr, [^{3}H]thymidine (0.5 μCi/well) was added to each well and the cells were incubated for a further 18 hr. The cells were then harvested onto glass fibre filters, which were washed and dried. The radioactivity incorporated was measured by liquid scintillation counting.

Data analysis. All data are expressed as mean values ± SEM. The fatty acid composition of membrane phospholipids and data concerning the proliferative response of PBMC to Con A were analysed by the Student's *t*-test. The other data were analysed using the STATVIEW II program for Macintosh according to one-way or two-way analysis of variance with balanced mixed models, as indicated in the legends of the figures. Calculated F values are given. They were considered as significant when the calculated P value was less than 0.05.

RESULTS

The preincubation of human PBMC with 5 μM EPA in the presence of 5 μM serum albumin induced a 2.7-fold increase of this FA in membrane phospholipids (in mol %: 0.31 ± 0.03 in control cells vs 0.85 ± 0.09 in EPA-treated cells, $N = 4$, $P < 0.001$), whereas the proportions of 22:5n − 3 and 22:6n − 3 were not significantly modified. These

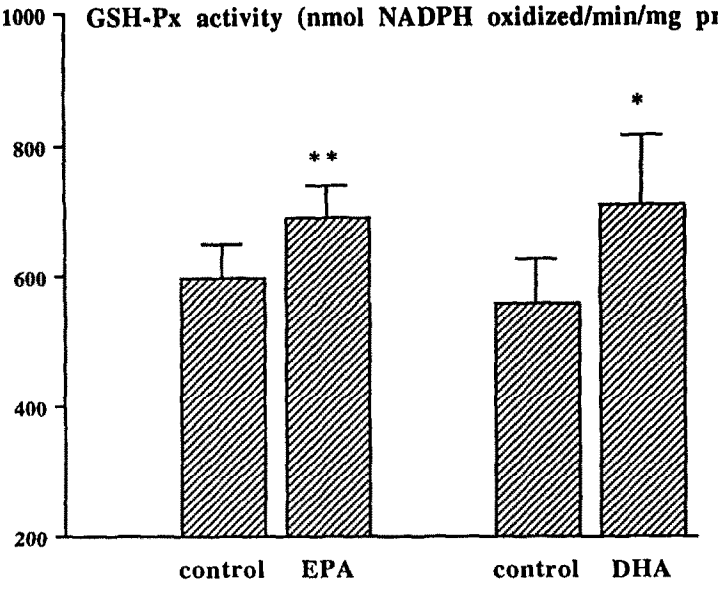

Fig. 1. Influence of EPA and DHA on the GSH-Px activity of human PBMC. Human PBMC were incubated in RPMI medium containing 1% human AB serum, in the absence (control) or presence of 5 μM EPA or DHA for 90 min at 37° (serum albumin to fatty acid ratio = 1). At the end of the incubation period, cells were lysed as described in Materials and Methods and GSH-Px activity was assayed in the 105,000 *g* supernatant fractions. Results are expressed in nmol NADPH oxidized/min/mg protein and are means ± SEM. Control/EPA: 13 experiments performed with PBMC from 13 different donors. Control/DHA: nine experiments performed with PBMC from nine different donors. Within one experiment, each determination was done in quadruplicate. For comparison in each group, data were evaluated by a two-way analysis of variance with a balanced mixed model: control/EPA: $F(1,12) = 12.97$, ** $P < 0.01$; control/DHA: $F(1,8) = 6.26$, * $P < 0.05$.

data suggest that human PBMC do not efficiently elongate 20:5n − 3, at least during the period of incubation considered (90 min). Similarly, the preincubation of PBMC with 5 μM DHA increased significantly (+65%) its content in phospholipids (in mol %: 2.84 ± 0.32 in control cells vs 4.70 ± 0.59 in DHA-treated cells, N = 4, $P < 0.04$). At the same time, the 22:5n − 3 and 20:5n − 3 contents were not significantly modified as compared to control cells. This result indicates that, in PBMC, 22:6n − 3 is not substantially retroconverted. Neither EPA nor DHA treatment of the cells significantly modified the phospholipid content of saturated (16:0, 18:0), monounsaturated (18:1n-9) or n − 6 FAs (not shown).

As shown in Fig. 1, the preincubation of human PBMC with 5 μM EPA or 5 μM DHA in the presence of 5 μM serum albumin significantly increased GSH-Px activity of the cytoplasmic compartment above that of control cells incubated in the presence of albumin alone ($P < 0.01$ and $P < 0.05$ for EPA and DHA, respectively). To determine whether this increase in GSH-Px activity was a specific effect of n − 3 FAs, PBMC were incubated in the presence of various FAs belonging to the saturated, n − 9 or n − 6 families. Saturated FAs such as palmitic (16:0) or stearic (18:0) acids as well as monounsaturated (18:1n − 9) and n − 6 polyunsaturated (18:2n − 6) acids only exhibited marginal effects on GSH-Px activity. Since the amplitude of GSH-Px activation induced by DHA and EPA might vary from experiment to experiment, the effect of these n − 3 FAs was also tested for comparison on aliquots of cells issued from the same donors. Only EPA and DHA were able to significantly increase GSH-Px activity whereas all the other fatty acids tested were inactive (Fig. 2).

In the above experiments the concentration of EPA and DHA (5 μM) was chosen to give a FA to serum albumin ratio of 1, which proves to be in the physiological range. Indeed, this molar ratio is lower than 2 under usual conditions of human physiology [19, 20]. To examine whether the influence of EPA and DHA on GSH-Px activity was dose dependent, the FA to serum albumin ratio was varied from 0.5 to 4 giving final FA concentrations of 2.5–20 μM. As shown in Fig. 3, the stimulatory effect of EPA and DHA on cytosolic GSH-Px activity was observed starting from the lowest FA to serum albumin ratio tested (0.5, 2.5 μM FA), remained significant for the ratio 1 (5 μM FA) and then declined for FA concentrations higher than 5 μM.

Among the numerous biochemical events involved in the mitogenic activation of lymphocytes, phospholipase A_2 activation is known to occur very early [21]. Such activation induces the release of free arachidonic acid and other PUFAs in the medium which might in turn modulate GSH-Px activity. In control PBMC incubated with serum albumin alone, Con A stimulation for a short period of time (30 min)

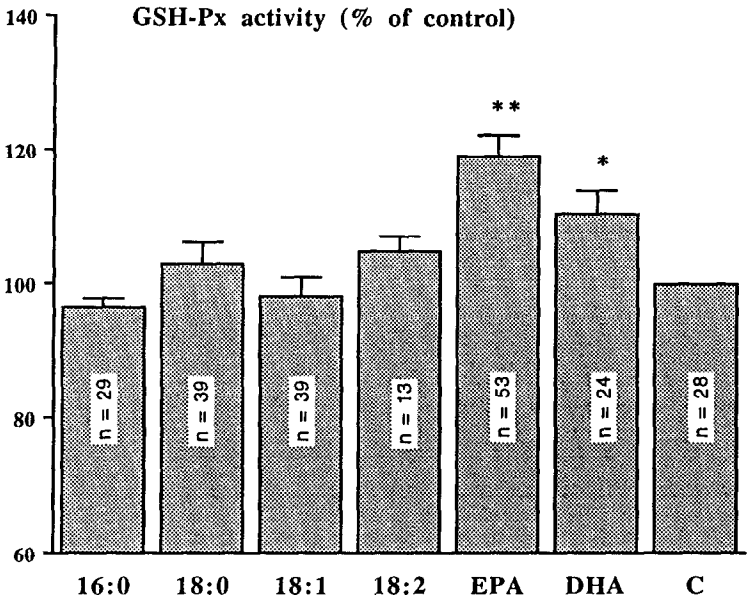

Fig. 2. Specificity of FA effects on the GSH-Px activity of human PBMC. Human PBMC were incubated in RPMI medium containing 1% human AB serum, in the absence (control) or presence of 5 μM of various FAs (palmitic: 16:0, stearic: 18:0, oleic, 18:1n-9, linoleic: 18:2n-6, EPA: 20:5n-3 and DHA 22:6n-3) for 90 min at 37° (serum albumin to FA ratio = 1). At the end of the incubation period, cells were lysed as described in Materials and Methods and GSH-Px activity was assayed in the 105,000 g supernatant fractions. Results are expressed in % of control values and are means ± SEM of two to four experiments performed with two different donors. Data were analysed by a one-way analysis of variance (ANOVA-1) and the level of statistical significance of the difference with control value was evaluated by the Fisher PLSD test. ** $P < 0.01$, * $P < 0.05$. The number of determinations are indicated on the graph.

did not significantly modify GSH-Px activity (Fig. 4). In contrast, in PBMC pretreated with 5 μM EPA plus 5 μM albumin, Con A significantly decreased GSH-Px activity to a level slightly lower than that observed with control cells. Similar significant results were observed in DHA-pretreated cells in spite of the greater variability of the measures (Fig. 4).

Since we have measured cytosolic GSH-Px activity in 105,000 g supernatant fractions, one hypothesis could be that the effect of n − 3 FAs and Con A that we have observed might be indirect and results from a different distribution of total proteins between the particulate and the cytosolic compartments. As shown in Fig. 5, neither EPA nor DHA modified such a distribution as compared with control cells incubated with albumin alone. Similarly, Con A stimulation did not modify this distribution, both in control cells and in EPA-treated cells but significantly increased the per cent of cytosolic proteins in DHA-treated cells. Although, this non-specific increased protein content of the cytosolic compartment might artificially give an underestimation of GSH-Px activity expressed per mg protein, it could not entirely explain the Con A-induced decrease in GSH-Px activity that we have observed in DHA-treated cells.

Another hypothesis to explain the EPA- and DHA-induced increase in GSH-Px activity might be that free FAs have a direct effect on GSH-Px activity of human mononuclear cells. We have therefore studied the influence of unesterified EPA and DHA upon the cytosolic GSH-Px activity of control PBMC when added directly in the enzyme assay medium. In these conditions, neither EPA nor DHA have a direct influence on GSH-Px activity at concentrations ranging from 1 to 50 μM (not shown).

The significant increase of GSH-Px activity obtained upon preincubation of PBMC with 5 μM EPA plus 5 μM albumin might result either from modifications of the kinetic properties of the enzyme or from an increased expression of the enzyme. The kinetic behavior of GSH-Px has been therefore investigated in the cytosolic fractions obtained from control cells incubated with albumin alone and from EPA-treated cells. Flohé *et al.* [22] have reported that the kinetic pattern of pure bovine GSH-Px can be well explained by a bireactant ping pong mechanism without any central enzyme–substrate complex formation according to the Dalziel equation:

$$[E_0]/v = \phi_1/[A] + \phi_2/[B]$$

(mechanism I, see the legend of Table 1).

As shown in Table 1, kinetic data of GSH-Px activity obtained either with control cells or with EPA-treated cells and analysed by a non-linear adjustment program were not well-fitted to the above equation as indicated by the high residual variance and the fact that residues were not randomly distributed (not shown). Kinetic data can be better explained by the following equation:

$$[E_0]/v = \phi_0 + \phi_1/[A] + \phi_2/[B]$$

(mechanism II, see the legend of Table 1)

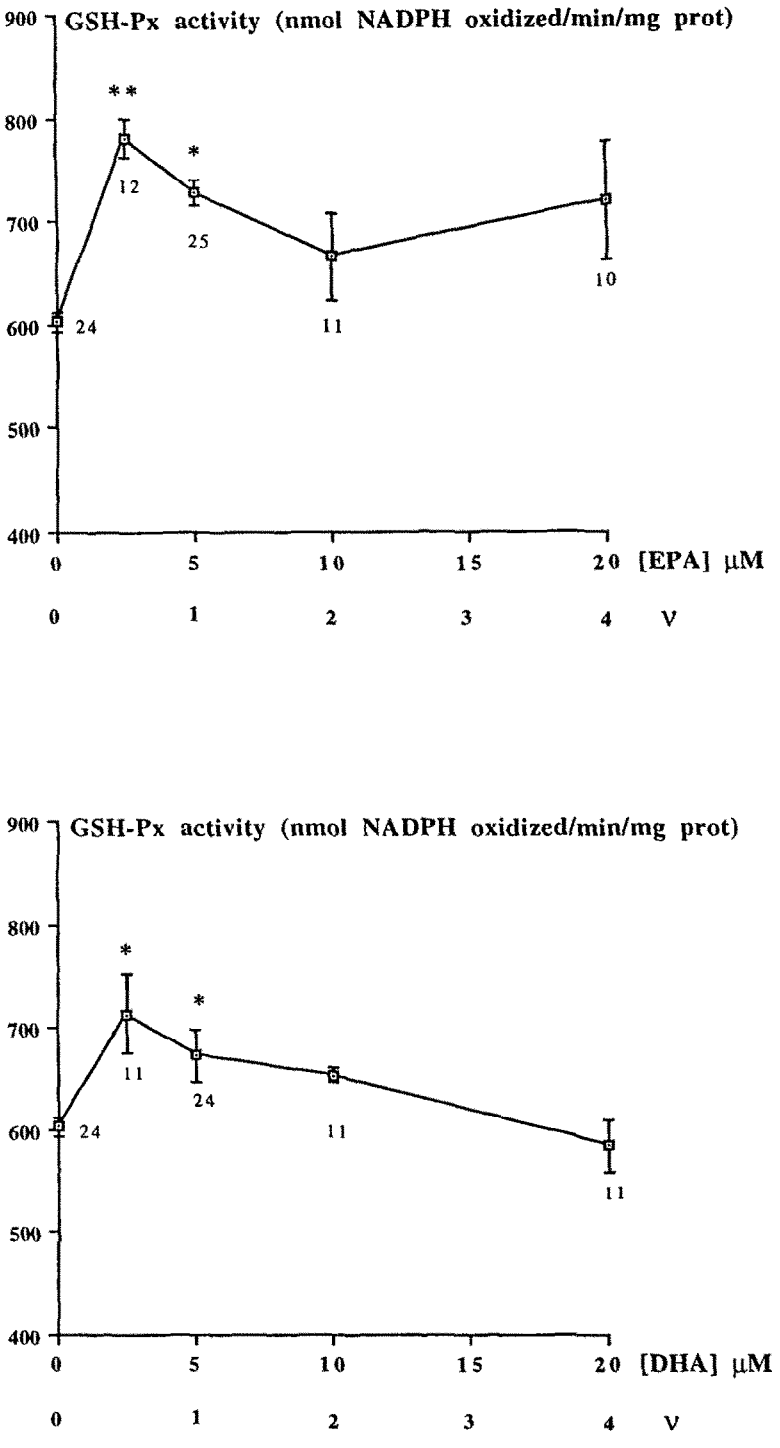

Fig. 3. Effects of increasing EPA and DHA concentrations on the GSH-Px activity of human PBMC. Human PBMC were incubated in RPMI medium containing 1% human AB serum, in the absence (control) or presence of 2.5 to 20 μM of EPA or DHA for 90 min at 37° (FA to serum albumin ratio from 0.5 to 4). At the end of the incubation period, cells were lysed as described in Materials and Methods and GSH-Px activity was assayed in the 105,000 *g* supernatant fractions. Results are expressed in nmol NADPH oxidized/min/mg protein and are means ± SEM of two experiments performed with two different donors. Data were analysed by a one-way analysis of variance (ANOVA-1) and the level of statistical significance of the difference with control value was evaluated by the Fisher PLSD test. ** $P < 0.01$, * $P < 0.05$. The number of determinations are indicated on the graph. v: FA to serum albumin ratio.

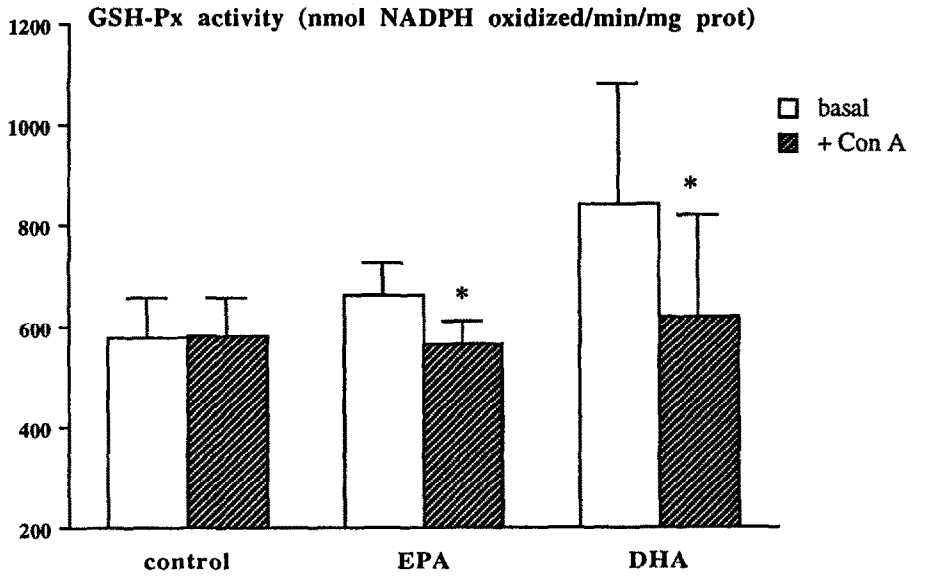

Fig. 4. Influence of Con A stimulation on the GSH-Px activity in control human PBMC and in EPA- and DHA-treated cells. Human PBMC were incubated in RPMI medium containing 1% human AB serum, in the absence (control) or presence of 5 μM EPA or DHA for 60 min at 37° (albumin to FA ratio = 1). Then, 5 μg of Con A per 10^6 cells were added and cells were incubated further for 30 min at 37°. Unstimulated cells (clear bars) were incubated for the same period of time without Con A. At the end of the incubation period, cells were lysed as described in Materials and Methods and GSH-Px activity was assayed in the 105,000 g supernatant fractions. Results are expressed in nmol NADPH oxidized/min/mg protein and are means ± SEM. Control: eight experiments performed with PBMC from eight different donors. EPA: eight experiments performed with PBMC from eight different donors. DHA: four experiments performed with PBMC from four different donors. Within one experiment, each determination was done in quadruplicate. The influence of Con A in each group was evaluated by a two-way analysis of variance with a balanced mixed model: control: unstimulated vs stimulated, $F(1,7) = 0.3$, NS; EPA: unstimulated vs stimulated, $F(1,7) = 7.4$, * $P < 0.05$; DHA: unstimulated vs stimulated, $F(1,3) = 12.1$, * $P < 0.05$.

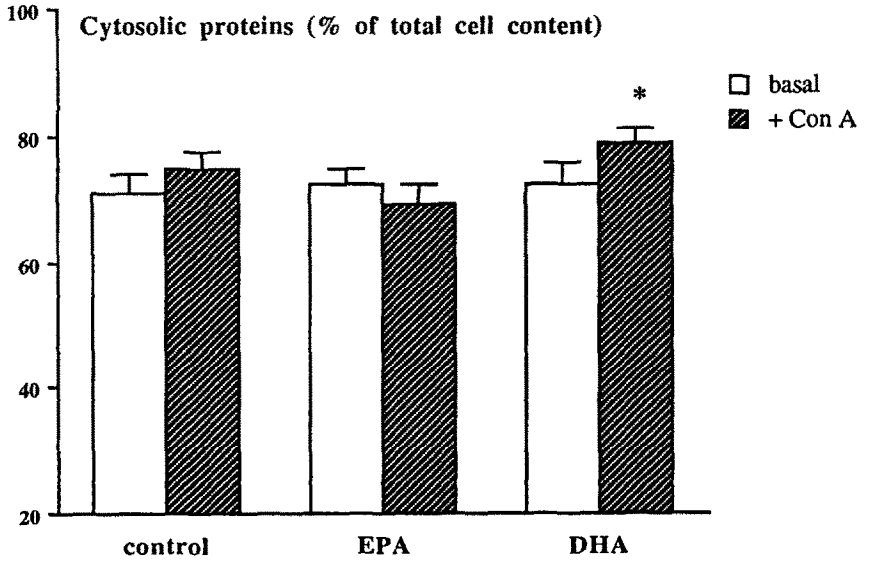

Fig. 5. Influence of EPA, DHA and Con A stimulation on the distribution of total proteins between the cytosolic and the particulate compartment after lysis and homogenization of human PBMC. Experiments were conducted as described in the legends of Figs 1 and 4. Cytosolic proteins were expressed as per cent of the total protein content of the cells and results are means ± SEM. Numbers of experiments are the same as in Fig. 4. Within one experiment, each determination was done in triplicate. The influence of Con A in each group was evaluated by a two-way analysis of variance with a balanced mixed model: control: unstimulated vs stimulated, $F(1,7) = 1.86$, NS; EPA: unstimulated vs stimulated, $F(1,7) = 0.45$, NS; DHA: unstimulated vs stimulated, $F(1,3) = 31$, * $P < 0.05$.

Table 1. Kinetic coefficients for the GSH-Px activity of control PBMC and of EPA-treated cells

Mechanism I	ddl	residual variance		$\phi_1/E_0(\times 10^3)$	ϕ_2/E_O $(\times 10^3)$	ϕ_1/ϕ_2
Control	81	649,420		0.440 ± 0.046	2.08 ± 0.29	0.2115 ± 0.0094
EPA	90	876,639		0.404 ± 0.043	2.05 ± 0.27	0.1971 ± 0.0089
Mechanism II	ddl	residual variance	E_0/ϕ_0	ϕ_1/ϕ_0	ϕ_2/ϕ_0	ϕ_1/ϕ_2
Control	80	209,838	2101.2 ± 318.3	0.508 ± 0.134	3.467 ± 0.730	0.1465 ± 0.0124
EPA	89	218,061	1944.6 ± 236.2	0.397 ± 0.091	3.034 ± 0.531	0.1306 ± 0.0102

Kinetic data were analysed using a computerized non-linear curve fitting program AJNL for Macintosh according to two different models.

Model I from Flohé *et al.* [22]:

$$E + A \underset{k+1}{\longrightarrow} F + B \underset{k+3}{\longrightarrow} G + B \underset{k+3}{\longrightarrow} E$$

$[E_0]/v = \phi_1/[A] + \phi_2/[B]$ where $\phi_1 = 1/k + 1$ and $\phi_2 = 1/k + 2 + 1/k + 3$, with $k + 1$ = velocity constant of the forward reaction peroxide/enzyme, $k + 2$ and $k + 3$= velocity constants for the interaction of the first and the second molecules of GSH with the enzyme, respectively.

Model II from Dalziel [23]:

$$E + A \underset{k_1}{\longrightarrow} EA \underset{k_3}{\longrightarrow} E' + R$$

$$E' + B \underset{k_5}{\longrightarrow} E'B \underset{k_7}{\longrightarrow} E'' + Q$$

$$E'' + B \underset{k_9}{\longrightarrow} E''C \underset{k_{11}}{\longrightarrow} E + P$$

$[E_0]/v = \phi_0 + \phi_1/[A] + \phi_2/[B]$ where $\phi_0 = 1/k_3 + 1/k_7 + 1/k_{11}$, $\phi_1 = 1/k_1$, $\phi_2 = 1/k_5 + 1/k_9$.

In both models: E_0 = total enzyme concentration, [A] = *t*-butylhydroperoxide concentration and [B] = GSH concentration; [A]: 0.1–0.8 mM; [B]: 0.5–6 mM. Kinetic parameters were obtained from five separate kinetic experiments performed with three different donors.

as proposed by Dalziel for a triple-transfer mechanism [23]. The presence of a constant term as ϕ_0 might indicate that at least one of the reaction steps involves the formation of a central complex between the enzyme and the substrate. Since the actual enzyme concentration E_0 is not known, the absolute values of ϕ_0, ϕ_1 and ϕ_2 are not directly available and can only be estimated as a ratio. EPA treatment of human PBMC did not significantly modify the various kinetic ratios suggesting that no drastic modification of the kinetic properties of the enzyme had occurred.

The proliferative response of PBMC to Con A stimulation was further investigated after 66 hr culture experiments (Fig. 6). Only DHA was able to significantly lower thymidine incorporation as compared to control cells incubated without added FAs whereas EPA was inactive.

DISCUSSION

Results of the present study show that a short incubation period (90 min) of human PBMC with 5 μM of either EPA or DHA was sufficient to induce a significant enrichment of cellular phospholipids in the corresponding FA. This enrichment was accompanied by an increased GSH-Px activity of the cells. DHA treatment of the cells also induced a significant decrease of the proliferative response to the mitogenic stimulation with Con A. A similar trend was observed for EPA. An important possibility to be excluded was that EPA and DHA were merely acting as non-specific detergents. However, this eventuality has to be ruled out since the stimulation of GSH-Px activity was observed with n − 3 FAs, but not with saturated, monounsaturated or n − 6 FAs (Fig. 2). Another interesting finding of the present study was that the stimulatory effect of both EPA and DHA was higher for the lowest concentration tested (2.5–5 μM) and then started to decline for n − 3 FA concentrations higher than 5 μM.

The potential mechanisms supporting the n − 3 FA-induced increase of GSH-Px activity are presently unknown and it is hard to link their incorporation in cell phospholipids with the increased GSH-Px activity observed. Their significant, but, moderate accumulation in phospholipids suggests they behaved as in response to *in vivo* dietary supplementation. Under our experimental conditions, it is likely that unesterified EPA or DHA content was low in resting cells. However, it has been suggested that Con A treatment stimulates the esterification of PUFAs in the phospholipid pool [15, 24]. Our results, showing that treatment with Con A attenuates the increased GSH-Px activity, might suggest that unesterified n − 3 FAs could be responsible for the enzyme stimulation observed, whether the active form was the FA or some of its oxidized derivative(s). Although free EPA and DHA were not able to directly modulate GSH-Px activity in cell-free preparations (results not shown), these FAs are

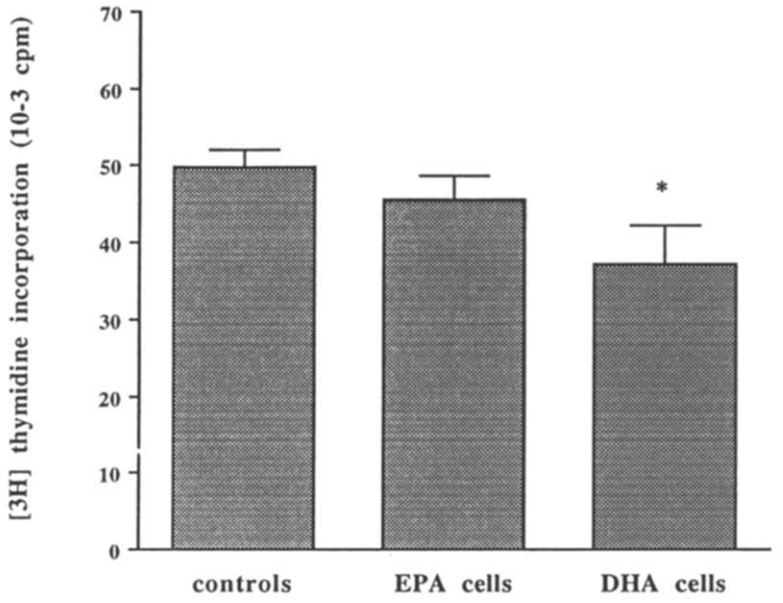

Fig. 6. Influence of EPA and DHA upon [^{3}H]thymidine incorporation into Con A-stimulated human PBMC. PBMC were cultured in RPMI + human AB serum previously incubated overnight at 37° with (5 μM EPA or DHA, albumin/FA = 1) or without FAs, in the presence or absence of 5 μg/mL Con A. After 48 hr, [^{3}H]thymidine was added and the cells were incubated further for 18 hr, prior to harvesting and radioactivity measurements. Data are expressed as Δ cpm (cpm with Con A − cpm without Con A) and are means ± SEM of eight determinations. Statistical significance vs controls without FA: * $P < 0.05$.

effective when added to intact cells in culture. They might, for example, improve the utilization of selenium present in the human serum. Perona *et al.* [9] have shown that, *in vitro*, selenium could raise GSH-Px activity when added to erythrocytes. EPA was not able to modify the kinetic parameters of the enzyme which favors the hypothesis of an increased expression during the preincubation period.

There have been several reports on the *in vitro* effects of various FAs upon antigen or mitogen-stimulated proliferation of lymphocytes in culture. Generally these studies have shown a decreased response to lectins in the presence of FAs, although the extent of the inhibition varied between studies. In rat lymph node lymphocytes treated with a relatively high concentration of FAs (33 μM), Calder *et al.* [25] have pointed out a higher suppressive effect for EPA (−80%) than DHA (−60%). These authors have provided further evidence that the inhibition of lymphocyte proliferation by FAs might be independent of eicosanoid production in rat as well as in human lymphocytes [26, 27]. In the present study, DHA was more effective than EPA in decreasing the proliferative response of PBMC to Con A activation whereas its enhancing effect on GSH-Px activity was more variable than EPA effect. Several hypotheses might be envisaged. It is known that certain aspects of the T-cell response require the action of active oxygen derivatives while other aspects require the action of antioxidants such as cysteine and GSH [28]. Thus, the strength of an immunological response depends decisively on an operational range defined, on the one hand, by the strength of the prooxidant state, and on the other hand, by the strength of T-cells to shift to an antioxidant state. In cells treated with 5 μM EPA, the increase in GSH-Px activity might be sufficient to maintain an adequate peroxide tone and to favor the proliferative state. It is noteworthy that, in studies reporting an inhibitory effect of EPA on the lymphoproliferative response, doses in the 30–250 μM range have been used [25–27]. Results of the present study have shown that low doses of EPA were more effective to stimulate GSH-Px activity than high doses. Thus, in the experimental conditions reported by Calder *et al.* [25–27], the level of hydroperoxides derived from EPA is very likely to be higher than in our experimental conditions. In contrast, in DHA-treated cells, the increase in GSH-Px activity might be unable to scavenge all lipid hydroperoxides so that the equilibrium would be in favor of an antiproliferative state. Indeed, DHA has been shown to be more sensitive to lipid peroxidation and to produce higher malonaldehyde quantities than EPA [29]. Although increased GSH-Px activity may play a role, the effect of n − 3 FAs upon lymphocyte proliferation is very likely to involve more complex mechanisms.

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