Effects of Different Dietary Oils on Inflammatory Mediator Generation and Fatty Acid Composition in Rat Neutrophils

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Virgin olive oil (VOO) compared with fish oil (FO) and evening primrose oil (PO) on the ability of stimulated leukocytes to produce inflammatory mediators was investigated in rats. Weaned Wistar rats were fed a basal diet (BD) (2% by weight of corn oil) or diets containing 15% by weight of VOO, PO, or FO. After 8 weeks, glycogen-elicited peritoneal polymorphonuclear leukocytes, mainly neutrophils, were isolated. The calcium-ionophore stimulated neutrophils (2.5×10^6 cells/mL) obtained from rats fed the different oils produced a higher release of lysosomal enzymes (β-glucuronidase, lysozyme, and myeloperoxidase [MPO]) compared with those fed BD. The production of reactive oxygen species (ROS) in response to the stimulant, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), by neutrophils from the VOO group (15.44 nmol of O_2^- and 6.56 nmol of H_2O_2) was similar to the BD group (12.01 nmol O_2^- and 8.49 nmol H_2O_2) and significantly lower than the PO (20.90 nmol O_2^- and 10.84 nmol H_2O_2) and FO (20.93 nmol O_2^- and 12.79 nmol H_2O_2) groups. The cyclooxygenase-derived eicosanoid production was reduced by the lipid enrichment of the diets. Whereas the generation of prostaglandin E_2 (PGE_2) was significantly decreased in VOO (5.40 ng/mL), PO (4.95 ng/mL), and FO (1.44 ng/mL) groups compared with BD (8.19 ng/mL), thromboxane B_2 (TXB_2) reduction was especially significant in neutrophils from the FO diet group (14.67 ng/mL compared with 26.69 ng/mL from BD). These experimental data suggest that FO and PO, as well as VOO, could be considered a valuable strategy in preventing the generation of some inflammatory mediators.

EPIDEMIOLOGIC AND experimental data indicate that changes in the source of lipid consumed in the diet may modify the fatty acid composition of many cell types, including those involved in the development of many inflammatory and immunologic diseases.[1-4] Although in recent years there has been a great interest in the immunomodulatory effects of n-3 polyunsaturated fatty acids (PUFA), less attention has been paid to n-6 and n-9 fatty acids, such as gammalinolenic and oleic acids, respectively.

Eicosapentaenoic (EPA, 20:5, n-3) and docosahexaenoic (DHA, 22:6, n-3) acids, from fish oil (FO), are known to be beneficial in the treatment of cardiovascular diseases,[5] rheumatoid arthritis,[6,7] and even in psoriasis.[8] It has been postulated that the beneficial effect of FO is mediated by changes in eicosanoid production, leading to the formation of less biologically active substances. Indeed, n-3 PUFA have been reported to reduce prostaglandin E_2 (PGE_2), thromboxane A_2 (TXA_2), and leukotriene B_4 (LTB_4) production, with a concomintant increase in the levels of PGE_3, TXA_3, LTB_5, nonvasoconstrictory, nonproaggregatory, and weakly chemotactic agents, respectively.[9] Classical studies have demonstrated that these diets can decrease inflammation processes and are immunosupressive in vivo.[10,11]

The dietary essential fatty acid linoleic acid (LA) is metabolized within the body, giving rise to gamma linolenic acid (GLA, 18:3, n-6) and dihomo-γ-linolenic acid (DGLA, 20:3, n-6), which turns into prostaglandins of the 1-series via the cyclooxygenase pathway. The use of evening primrose oil (PO) as a source of GLA has been investigated, and several clinical applications on arthritis and inmunologic diseases have been described.[12-15]

The Mediterranean diet, rich in monounsaturated fatty acids (MUFA), especially oleic acid from olive oil, has been associated with a lower risk of coronary heart disease. The consumption of diets rich in MUFA has been linked to a low prevalence of atherosclerosis,[16] and there has been great interest in their effects on lipoprotein metabolism.[17] It has been suggested that fatty acids of the n-9 series derived from the oleic acid might play an important role in the immunomodulatory processes.[18,19] Therefore, because virgin olive oil (VOO) contains other specific components, we have developed several investigations to determine the possible influence of these compounds on its effects. In this regard, we have demonstrated that olive oil phenolics reduce in vitro the generation of reactive oxygen species (ROS) from phagocytic cells and inhibit the activity of the 5-lipoxygenase enzyme that is involved in proinflammatory events[20]) and also prevent damage by reactive nitrogen species.[21] Moreover, in a recent study, we have observed that a high-phenolic VOO exhibited protective effects in two experimental models of inflammation in rats, in addition to an improvement in the loss of weight associated with the inflammatory process.[22]

The aim of the present study was to examine the influence of VOO on the production of inflammatory mediators by rat neutrophils, comparing the response with those from rats fed diets supplemented with PO and FO, well-known by their beneficial effects on inflammatory processes.

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Table 1. Fatty Acid Composition of Dietary Oils (% of total fatty acids)

Fatty Acids	Corn Oil*	VOO	PO	FO
14:0	0.01	—	0.04	0.66
16:0	10.36	11.80	6.44	24.11
16:1(n−7)	0.12	0.90	0.11	11.49
17:0	—	0.40	—	—
18:0	2.36	2.80	1.52	4.54
18:1(n−9)	33.05	79.2	14.16	15.59
18:1(n−7)	1.15	—	1.25	4.75
18:2(n−6)	50.89	3.50	68.85	2.98
18:3(n−3)	0.71	0.60	0.33	0.60
18:3(n−6)	0.43	—	6.44	0.58
18:4(n−3)	—	—	—	3.34
20:0	—	0.30	0.04	—
20:1(n−9)	0.22	0.20	0.18	2.13
20:2(n−6)	0.03	—	0.06	0.15
20:3(n−6)	—	—	0.04	0.17
20:4(n−6)	—	—	0.04	0.93
20:5(n−3)	—	—	—	18.61
22:4(n−6)	0.11	—	0.08	0.88
22:5(n−3)	—	—	—	2.84
22:6(n−3)	—	—	—	5.64
24:0	—	0.40	—	—
SFA	12.73	15.70	8.04	29.31
MUFA	34.64	80.20	15.70	33.96
PUFA	52.17	4.10	75.84	36.72

*Included in BD group only in 2% of total compared with the other dietary groups with 15% oil.

MATERIALS AND METHODS

Animals and Diets

Male Wistar rats (Centro de Instrumentación Científica, Granada, Spain), aged 3 weeks and weighing 60 g, were placed in cages with wire-net floors in a controlled room (on a 12-hour light:dark cycle; temperature, 22°C ± 2°C; humidity 70% to 75%). The rats were randomly divided into 4 groups of 6 to 8 animals and had free access to food and water; their food intake and growth were monitored at regular intervals. Each group was fed for 8 weeks a purified diet[23] containing sucrose 50%, casein 20%, corn starch 15%, alphacel 5%, DL-methionine 3%, and choline chloride 2%, which was purchased from Musal & Chemical (Granada, Spain) and AIN-93 mineral mix 3.5% and AIN-93 vitamin mix 1%, purchased from ICN Nutritional Biochemicals (Cleveland, OH). The basal diet (BD) contained approximately 2% by weight from corn oil (BD group). The lipid-enriched diets contained 15% by weight from extra VOO (VOO group) Picual variety; 15% by weight from PO (PO group) obtained by hexanic extraction from *Oenothera biennis* seeds or 15% by weight from sardine oil (FO group) from AFAMSA (Vigo, Spain). To minimize oxidation, all diets were prepared twice weekly and stored at 4°C under a N_2 atmosphere. The fatty acid composition of the oils was determined as previously described[24] and is shown in Table 1.

Reagents. All reagents and chemicals were of analytical grade from Sigma-Aldrich Química (Madrid, Spain).

Preparation of Rat Peritoneal Mixed Leukocytes

A suspension of leukocytes containing approximately 85% polymorphonuclear leukocytes (PMNs) and 15% mononuclear cells was elicited from male Wistar rats fed for 8 weeks (260 to 280 g) by an intraperitoneal (IP) injection of a 10-mL solution of 6% oyster glycogen in saline[25] followed 16 to 20 hours later by 60 mL ice-cold modified Hank's balanced salt solution (HBSS) free of Ca^{2+} and Mg^{2+}. After about 90 seconds of a vigorous massage, the peritoneal washing was removed, centrifuged at 400 × *g* for 10 minutes at 4°C and the contaminating erythrocytes in the pellet lysed after resuspension in a small volume of 0.2% saline for 30 seconds, after which isotonicity was re-established by adding excess HBSS. After a further centrifugation and washing, the mixed peritoneal leukocytes were resuspended in complete HBSS at 2.5×10^6 cells/mL containing 1.26 mmol/L Ca^{2+} and 0.9 mmol/L Mg^{2+}. Cell viability based on trypan blue exclusion was greater than 95%.

Assay of MPO, β-Glucuronidase, and Lysozyme Enzymes Released From Stimulated Rat Peritoneal Leukocytes

Triplicate aliquots of 0.5 mL leukocytes were preincubated at 37°C for 10 minutes and its stimulation was performed adding 5 μL calcium ionophore A23187 in dimethyl sulfoxide (DMSO) to give a final concentration of 1 μmol/L for a further 10 minutes of incubation. Control tubes contained unstimulated or treated cells with 5 μL 20% Triton X-100 detergent to disrupt the cells for measuring total enzyme content. The cells were pelleted by centrifugation at 2,500 × *g* for 10 minutes at 4°C, and the supernatants were decanted and frozen for assay of the lysosomal enzymes. The assays of released lysozyme and β-glucuronidase were performed using 4-methylumbelliferyl-β-D-glucuronide and *Micrococcus lysodeikticus* as substrates, respectively.[26] MPO release was determined in triplicate by measuring the rate of oxidation of *o*-dianisidine,[27] adapting the concentrations for measurement in a microplate reader at 450 nm. The total MPO, β-glucuronidase, and lysozyme content was measured by performing equivalent tests on neutrophil supernatants of the detergent-treated cells (see above), and the results for enzyme release of each group are expressed as a percentage of this total amount of enzyme.

Measurement of ROS Generated From Activated Rat Peritoneal Leukocytes

Superoxide secretion in response to 12-O-tetradecanoylphorbol-13-acetate (TPA) was measured by oxidation of reduced cytochrome *c*.[28] Triplicate aliquots of 1 mL leukocytes were preincubated at 37°C for 10 minutes, and 100 μL cytochrome *c* at 80 μmol/L was added. After 10 minutes, the stimulation was performed adding 10 μL TPA in DMSO to give a final concentration of 1 μmol/L, or both TPA and superoxide dismutase (SOD, superoxide oxidoreductase EC 1.15.1.1.), (control-SOD) during 15 minutes; control tubes contained unstimulated cells. The cells were pelleted by centrifugation at 2,500 × *g* for 10 minutes at 4°C, the supernatants were decanted, and the absorbance was read at 550 nm in a Perkin Elmer (Wellesley, MA) spectrophotometer Lambda 3. Superoxide concentrations were calculated using the molar extinction coefficient of oxidized cytochrome *c* (2.1×10^4 $mol/L^{-1} \cdot cm^{-1}$).

Hydrogen peroxide secretion by neutrophils in response to stimulation by TPA was measured by oxidation of phenol red in the presence of peroxidase.[29] Triplicate aliquots of 1 mL leukocytes containing 10 μL phenol red (28 mmol/L) and 10 μL horseradish peroxidase (type II from Sigma) (50 μg/mL) were preincubated at 37°C for 5 minutes and the stimulation was performed adding 10 μL TPA in DMSO to give a final concentration of 1 μmol/L, or both TPA and catalase (oxidoreductase EC 1.11.1.6.) (control-catalase) for 30 minutes. Control tubes contained unstimulated cells. The cells were pelleted by centrifugation at 2,500 × *g* for 5 minutes at 4°C, the supernatants were decanted, and 10 μL NaOH 1N was added. The absorbance was read at 610 nm in a Perkin Elmer spectrophotometer Lambda 3. Hydrogen peroxide concentrations were calculated by comparision to a standard curve.

Stimulation of the Release of Prostanoids and Its Immunoassay

Triplicate aliquots of 0.5 mL leukocytes were preincubated at 37°C for 10 minutes and its stimulation was performed by adding 5 μL calcium ionophore A23187 in DMSO to obtain a final concentration of 1 μmol/L for a further 10 minutes of incubation. Control tubes contained no cells or cells incubated with the vehicle on its own (DMSO) or treated with the COX inhibitor, indomethacin, at a final concentration of 20 μmol/L (control-indomethacin). The cells were pelleted by centrifugation at 2,500 $\times$ *g* for 10 minutes at 4°C, and the supernatants were decanted and frozen at −20°C and the measurements performed in less than 3 months. For the PGE_2 assay, aliquots (10 μL) of the thawed samples were subjected to radioimmunoassay[25,26] (radioactive products from Amersham, Little Chalfont, UK) and the bound dpm counted in a Packard model 1900TR liquid scintillation analyzer (Packard Instrument Co, Downers Grove, IL). For the thromboxane B_2 (TXB_2) assay, aliquots (10 μL) of the thawed samples were subjected to commercially available thromboxane B_2 enzyme immunoassay kit, purchased from Cayman Chemical (MI),[30] and the measurements were performed according to instructions, reading at 414 nm in a Labsystem (Helsinki, Finland) Multiskan EX Prymary EIA V.2.1.

Extraction of Lipids and Fatty Acid Analysis From Rat Peritoneal Leukocytes

Extraction of total lipids from polymorphonuclear leukocytes (1.5 to 2 $\times$ 10^8 cells /mL) was performed following the method of Folch et al[31] in the presence of butylated hydroxytoluene (BHT, 2% wt/vol) as antioxidant. The fatty acid composition of neutrophils was determined by gas chromatography as previously described.[32] In brief, the samples were saponified by heating for 25 minutes with 5 mL 0.2 mol/L sodium methylate and heated again at 80°C for 25 minutes with 6% H_2SO_4 in anhydrous methanol (vol/vol). The fatty acid methyl esters formed were recovered with hexane and analyzed in a Hewlett-Packard 5890 series II gas chromatograph equipped with flame ionization detector, using an Omegawak 320 fused silica capillary column (30 m $\times$ 0.32 mm internal diameter, 0.25 mm film). The initial column temperature was 200°C, which was held for 10 minutes, then programmed from 200°C to 230°C at 2°C/minute.

Statistical Methods

All results were subjected to 1-way analysis of variance (ANOVA) followed by the 2-tailed Student's *t* test and represent means ± SEM of cells from 6 animals per group.

RESULTS

No significant differences in food consumption or growth rates due to diet were observed in any of the 4 dietary groups of rats. The final body weights were 403 ± 9 g for animals of the BD group, 395 ± 15 g for rats fed the VOO diet, 413 ± 7 g for rats fed the PO diet, and 408 ± 13 g for rats fed the FO diet.

The calcium-ionophore-stimulated neutrophils from all groups fed oil-rich diets produced an increment in the lysosomal enzymes release (lysozyme, β-glucuronidase, and MPO) compared with the BD group (Fig 1). The lysozyme and β-glucuronidase–induced secretion were higher after consumption of all oil-rich diets compared with BD. However, the degranulation of azurophilic granules represented by the enzyme MPO was only significantly higher in FO ($P < .01$) and PO ($P < .001$) groups compared with BD, but not in the VOO group.

The modification on neutrophil ROS generation by the oil-enriched diets in response to 15 or 30 minutes of TPA-stimulation is shown in Fig 2. The production of superoxide (O_2^-) and hydrogen peroxide (H_2O_2) by 2.5 $\times$ 10^6 cells was 12.01 and 8.49 nmol, respectively, for the BD group, not being significantly different from the production of cells after VOO (15.44 nmol of O_2^- and 6.56 nmol of H_2O_2). On the contrary, higher releases of these species were detected in neutrophils from the PO (20.90 nmol O_2^- and 10.84 nmol H_2O_2) and FO (20.93 nmol O_2^- and 12.79 nmol H_2O_2) dietary groups.

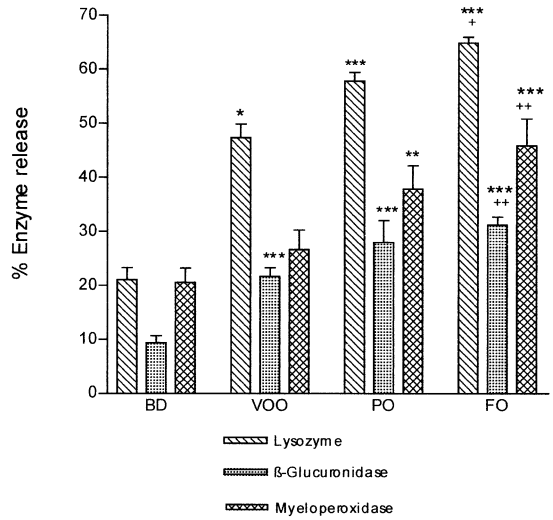

Fig 1. Percent of total enzyme release by ionophore-activated rat peritoneal leukocytes isolated from rats fed on different oil-enriched diets. Data are means ± SEM for 6 animals fed on each diet; *$P < .05$, **$P < .01$, ***$P < .001$ *v* BD; +$P < .05$, ++$P < .01$ *v* VOO.

The generation of the cyclooxygenase-derived eicosanoids was reduced by dietary lipid manipulation (Fig 3). After calcium ionophore stimulation, the release of PGE_2 was significantly reduced in neutrophils (2.5 $\times$ 10^6 cells) from rats fed FO (1.44 ng/mL), PO (4.95 ng/mL), and VOO (5.40 ng/mL) compared with the basal diet group (8.19 ng/mL). Similarly, the concentrations of TXB_2 were decreased compared with the BD group (26.69 ng/mL) in the medium of neutrophils from rats fed FO (14.67 ng/mL), PO (20.83 ng/mL), and VOO (23.14 ng/mL) diets, although the reduction was only significant for the FO group.

The neutrophil fatty acid composition is shown in Table 2. As it was expected, oleic acid was the main fatty acid incorporated in the cells isolated from rats fed VOO. In this and the FO groups, the content of arachidonic acid (AA) (20:4 (n-6) was lower compared with the BD group (7.25% and 3.24%, respectively *v* 12.93%). However, the differences between the PO and BD groups were mainly found in the higher incorporation of LA (18:2 [n-6]), gamma linolenic acid (GLA) (18:3 [n-6]), and dihomo-gammalinolenic acids (DGLA) (20:3 [n-6]) and not in the AA content. The n-3 PUFA, EPA (20:5 [n-3]), docosapentaenoic acid (DPA) (22:5 [n-3]), and DHA (22:6 [n-3]) were mainly incorporated into neutrophils from the FO group. These results yielded a reduced AA/LA ratio for leukocytes from rats fed PO and a lower n-6/n-3 ratio for cells from those fed FO. Administration of both n-9 and n-3 diets produced a

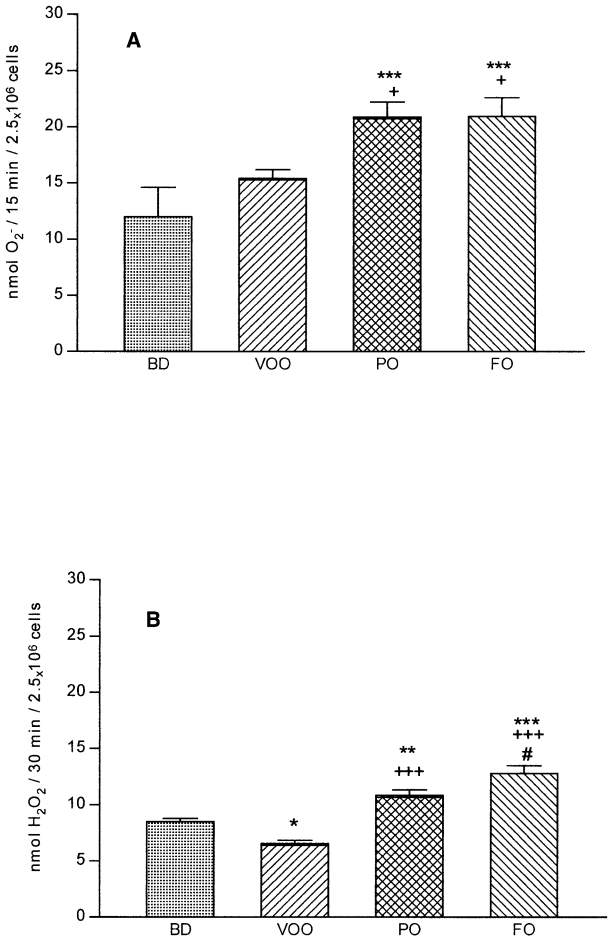

Fig 2. ROS generated by TPA-activated rat peritoneal leukocytes isolated from rats fed on different oil-enriched diets. (A) Control: unstimulated cells, 2.04 nmoles O_2^-/2.5 × 10^6 cells and Control-SOD: stimulated cells plus SOD, 2.94 nmoles O_2^-/2.5 × 10^6 cells. (B) Control: unstimulated cells, 1.18 nmoles H_2O_2/2.5 × 10^6 cells and Control-CATALASE: stimulated cells plus CATALASE, 1.12 nmoles H_2O_2/2.5 × 10^6 cells. Data are means ± SEM for 6 animals fed on each diet; *P < .05, **P < .01, ***P < .001 *v* BD; +P < .05, +++P < .001 *v* VOO; #P < .05 *v* PO.

significant decrease in AA concentration compared with the n-6 enriched diet.

DISCUSSION

Numerous studies have described that dietary fatty acids are involved in the modulation of the immune system through mechanisms that modify the immune response.[33,34] The effects of dietary lipids on lymphocyte and macrophage functions are well established,[35,36] but the effects on neutrophil function present more discrepancies.[37]

Activated neutrophils secrete a variety of products including ROS, lysosomal enzymes, and eicosanoids, which play important roles in key neutrophil functions, such as the killing of microbial cells and the regulation of the inflammatory response. The relationship between fatty acid structure and biologic response has already been examined in specific and azurophil granules from human neutrophils incubated with different physiologic concentrations of fatty acids. In general, it has been found that as the number of double bonds in the fatty acid increases, so does its ability to stimulate the secretion of the lysosomal enzymes. In addition, a further increase is observed with increasing carbon chain length. The position of the double bounds is also critical, as less stimulation has been found for the 18- and 20-carbon fatty acids of the n-3 series than with their n-6 isomers.[38,39] In our study, the significant increase in lysozyme, β-glucuronidase, and MPO releases in leukocytes from PO and FO groups might be related by the higher incorporation of PUFA into the neutrophil membranes, mainly n-6 PUFA after PO and n-3 PUFA after FO (Table 2). However, this was not the case for the cells obtained from rats fed VOO. It is very unlikely that the effect observed in the lysozyme and β-glucuronidase releases might be due to the oleic acid (18:1[n-9]), a fatty acid with lower potency to trigger the degranulation process in neutrophils.[40,41] We have previously demonstrated that VOO polyphenols provide protective effects in

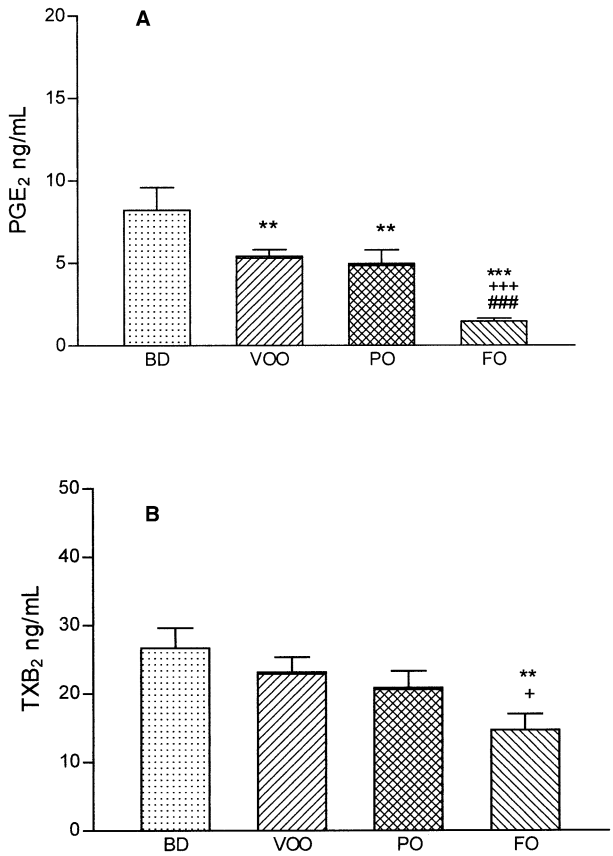

Fig 3. Generation of PGE_2 and TXB_2 by ionophore-activated rat peritoneal leukocytes isolated from rats fed on different oil-enriched diets. (A) Control: unstimulated cells, 0.42 ng PGE_2/mL and Control-INDO: stimulated cells plus 20 μmol/L COX inhibitor indomethacin,0.8 ng PGE_2/mL. (B) Control: unstimulated cells, 2.01 ng TXB_2/mL and Control-INDO: stimulated cells plus 20 μmol/L COX inhibitor indomethacin,3.50 ng TXB_2/mL. Data are means ± SEM for 6 animals fed on each diet; **P < .01, ***P < .001 *v* BD. +P < .05, +++P < .001 *v* VOO; ###P < .001 *v* PO.

Table 2. Fatty Acid Composition of Peritoneal Leukocytes (% of total fatty acids)

Fatty Acid	BD	VOO	PO	FO
14:0	1.56 ± 0.05	2.02 ± 0.17	1.80 ± 0.23	3.23 ± 0.63*
16:0	28.41 ± 0.49	31.68 ± 1.99	27.64 ± 0.19	28.23 ± 3.25
18:0	12.16 ± 0.10	16.02 ± 0.40	14.88 ± 0.15	19.68 ± 5.10*
18:1 (n-9)	27.04 ± 0.28	29.75 ± 0.43	17.08 ± 0.35‡	17.35 ± 1.85‡
18:1 (n-7)	5.93 ± 0.12	3.46 ± 0.32‡	2.94 ± 0.16‡	3.32 ± 0.20‡
18:2 (n-6)	8.39 ± 0.06	5.67 ± 0.75‡	14.73 ± 0.66‡	3.33 ± 0.12‡
18:3 (n-3)	0.16 ± 0.09	0.67 ± 0.03	<0.1	1.10 ± 0.07†
18:3 (n-6)	<0.1	<0.1	0.51 ± 0.02‡	<0.1
20:3 (n-6)	0.27 ± 0.16	0.29 ± 0.16	1.19 ± 0.26*	0.51 ± 0.24
20:4 (n-6)	12.93 ± 0.46	7.25 ± 1.31*	13.22 ± 0.42	3.24 ± 0.98†
20:5 (n-3)	<0.1	<0.1	<0.1	6.40 ± 0.59‡
22:5 (n-3)	1.60 ± 0.03	2.04 ± 0.23	1.62 ± 0.17	8.31 ± 1.59†
22:6 (n-3)	1.55 ± 0.05	1.15 ± 0.38	4.40 ± 0.85*	5.29 ± 1.03†
C20:4/C18:2	1.54	1.28	0.90	0.97
Total n-9 (%)	27.04 ± 4.64	29.75 ± 4.42	17.08 ± 2.74†	17.35 ± 2.90†
Total n-6 (%)	21.59 ± 1.69	13.21 ± 0.95†	29.65 ± 1.68*	7.08 ± 0.50‡
Total n-3 (%)	3.31 ± 0.25	3.85 ± 0.24	6.02 ± 0.72	21.10 ± 1.13†
n-6/n-3	6.52	3.43	4.92	0.33

NOTE. Values represent the mean ± SEM from 6 determinations.
Statistical significance: *$P < .05$, †$P < .01$, ‡$P < .001$ *v* BD.

experimental inflammation models.[22] In this regard, natural phenols, such as curcumin and capsaicin, can lower the secretion of some lysosomal enzymes.[42]

It is well established that neutrophils generate radical superoxide by activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in response to a wide range of stimuli through different transduction signal mechanisms.[43,44] Our results agree with those of other investigators in which stimulated macrophages showed a significant increase of superoxide and hydrogen peroxide in cells isolated from mice fed diets enriched in FO[35] and in n-3 PUFA, such a EPA and DHA.[45,46] The changes in fatty acid composition of the membrane phospholipids have great importance because of the alteration of plasmatic membrane characteristics as for example, fluidity. PUFA have been found to induce high levels of superoxide by human neutrophils and to induce morphologic changes (ie, evaginations), which could facilitate the production of endocytic vesicles and the phagocytosis process.[47] Such effects and the content in antioxidant polyphenols of VOO may explain the enhanced ROS production by neutrophils from rats fed FO and PO diets in our experiments compared with that of VOO and BD groups (BD contained only 2.5% by weight of dietary corn oil). Olive oil-supplemented diets have been recently observed to have an additional beneficial effect by increasing the $NO^{\cdot}/O_2^{-}$ ratio compared with FO supplementation, which increased both $NO^{\cdot}$ and O_2^{-}.[48] According to these investigators and our results, VOO might be more efficient in reducing oxidative stress than FO.

Dietary fatty acids also influenced the eicosanoid production by neutrophils ,as they may modulate the activity of enzymes involved in the synthesis of these substances, which participate as secondary messengers within the cytoplasm. We found that the levels of PGE_2 and TXB_2 were significantly more reduced in neutrophils from rats fed FO. Reduced amounts of these lipid mediators were also produced by the PO and VOO neutrophils, although their values were only significant for PGE_2 concentrations compared with those of the BD group. Neutrophils from FO-fed rats presented a lower concentration of AA, the major substrate for eicosanoid synthesis. The higher incorporation of EPA into these cells, reported as an inhibitor of AA conversion by cyclooxygenase, and the increase in DHA strong inhibitor of this enzyme, explain the significantly reduced release of PGE_2. On the other hand, the low AA content in leukocytes from VOO-fed rats, probably due to a reduced incorporation of its precursor LA, may be the reason for the lower level of AA metabolites, such as PGE_2 in this group.[49] Finally, the effect produced in the eicosanoid generation by the PO has been previously explained by the fact that GLA is converted to DGLA at a more rapid rate than DGLA is desaturated to AA; once DGLA is incorporated into cellular phospholipids, it may compete with AA for enzymes, including phospholipase A2, and prostaglandin G/H synthetase, resulting in the production of a less amount of PGE_2 and TXB_2.[50]

In summary, the present study shows that the nature of the dietary oils significantly affects the production level of secretory products by stimulated peritoneal polymorphonuclear leukocytes in rats. Despite that the most potent effect was observed in cells from rats consuming FO, PO also presented significant effects on some of the inflammatory parameters studied. In addition, our results provide important additional data about the modulating capability of the olive oil diet on the generation of crucial inflammation parameters: a lower increase in the production of dangerous ROS and proteolytic enzymes from neutrophils than FO or PO, but a moderate decrease in the generation of proinflammatory cyclooxygenase pathway-derived eicosanoids. These experimental data have confirmed the anti-inflammatory effects of FO and PO, but also suggest that VOO should be considered as a valuable strategy in modulating the generation of inflammatory mediators.

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