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Effect of dietary argan oil on fatty acid composition, proliferation, and phospholipase D activity of rat thymocytes

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Abstract

Objective: Argan oil is receiving increasing attention due to its potential health benefits in the prevention of cardiovascular risk, but no information to date is available about its possible effect on immune cells and functions.

Methods: To address this issue male rats were fed one of five diets that contained fish oil, argan oil, olive oil, coconut oil, or sunflower oil for 4 wk. The fatty acid composition of plasma and thymocyte lipids was then analyzed in relation to the mitogen-induced proliferation and phospholipase D (PLD) activity of thymocytes.

Results: The 18:2ω-6 proportion in thymocyte phospholipids from rats fed argan oil was significantly lower than that observed in phospholipids from rats fed sunflower oil and fish oil but higher than that found in the olive oil and coconut oil groups. Further, a significant positive linear relation was found between thymocyte proliferation and the 18:2ω-6 proportion in thymocyte phospholipids, whatever the diet. The proliferation response of thymocytes to mitogenic activation was also inversely correlated to PLD activity measured in intact thymocytes. Subsequent western blotting experiments indicated that the diet-induced variations in PLD activity mainly reflected variations in the expression of PLD2 protein.

Conclusions: On the whole, the present study shows that the effects of argan oil on immune cells are very similar to those of olive oil, and that, as a consequence, argan oil can be used as a balanced dietary supply without marked adverse effects on immune cell function.

Keywords: Dietary lipids; Oleic and linoleic acids; 18:2ω-6 proportion and thymocyte proliferation relation; Phospholipase D activity and thymocyte proliferation relation

Introduction

Argan oil obtained from the argan fruit of *Argania spinosa* represents about 25% of dietary fats consumed by the southwestern Moroccan population. It is characterized by its unique fatty acid composition [1,2]. This oil is particularly rich in oleic (45% to 48%) and linoleic (32% to 35%) acids and quite devoid of polyunsaturated fatty acids of the ω-3 family. It is currently used in traditional medicine for its cosmetic, bactericide, and fungicide properties. Several recent dietary studies have pointed out hypolipidemic, hypocholesterolemic, and antihypertensive effects in the rat [3,4]. Further, a clinical study conducted with southwest Moroccan subjects showed that a mean daily intake of 15 g of argan oil significantly decreases low-density lipoprotein cholesterol compared with non-consumers [5]. Dietary lipids may influence several aspects of lymphocyte function such as lymphocyte proliferation, lymphocyte-derived cytokine production, and cell-mediated immunity [6,7]. To date, the potential effect of argan oil consumption on lym-

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phocyte function has never been reported. Thus, we investigated whether feeding rats a diet enriched with argan oil would influence thymocyte proliferation. Because oleic and linoleic acids are the two main unsaturated fatty acids of argan oil, we also investigated the effect of olive and sunflower oils. Coconut oil, which is rich in saturated fatty acids, and sardine oil were used as sources of saturated and ω-3 polyunsaturated fatty acids, respectively, for comparison.

Dietary lipids modify the fatty acid composition of cell membrane and, more specifically, the composition of lymphocyte rafts, which are involved in the formation of an immunologic synapse [7,8]. These alterations may in turn influence a large variety of signaling pathways initiated by mitogenic activation. We previously reported that incorporation of docosahexaenoic acid (22:6ω-3) or 12-hydroxyeicosatetraenoic acid, in phospholipids of human lymphocytes markedly stimulates their phospholipase D (PLD) activity [9,10], and that this was accompanied by an inhibition of their proliferative response to mitogens [10,11]. PLD, which hydrolyzes the distal phosphodiester bound of phosphatidylcholine, has been described as a key regulator of cell proliferation in several cell models [12,13]. Two mammalian PLDs have been cloned from various human and murine sources. They are differently regulated by small G-proteins and protein kinase-C, but the activity of both enzymes is highly dependent on phosphatidylinositol bisphosphate [13]. Whereas PLD activation seems to favor cell proliferation in most cell types, this is not the case in human lymphocytes, where we observed opposite effects [9,10]. In addition, activation of a phosphatidylcholine-specific PLD has been shown to inhibit the proliferation of B lymphocytes [14]. Thus, in addition to their effects on thymocyte proliferation, we also investigated whether the different diets could influence PLD activity of rat thymocytes

Materials and methods

Animals and diets

All experimental procedures complied with the *Guide for the Care and Use of Laboratory Animals*. Male Sprague-Dawley rats weighing 140 to 200 g (mean 160.2 ± 2.7, n = 70) were housed individually at 22°C, with a 12-h light/dark cycle. They had free access to food and water. After 1 wk of acclimatization, animals were randomly assigned to one of five groups that differed only in the type of oil added to the fat-free semipurified diet (Usine d'Alimentation Rationnelle, Villemoisson-sur-Orge, France). Because fish and coconut oils must be supplemented with a minimal amount of linoleic acid to prevent essential fatty acid deficiency, 2 g of sunflower oil per 100 of g diet was added to each diet to standardize preparations. All diets were isoenergetic and provided the same amount of fat (10 g of oil/100 g of diet.).

Table 1
Composition of experimental diets*

Ingredient (g/100 g diet)	FO diet	AO diet	OO diet	CO diet	SO diet
Delipidated casein†	20.25	20.25	20.25	20.25	20.25
Cornstarch + glucose	57.0	57.0	57.0	57.0	57.0
Cellulose	5.50	5.50	5.50	5.50	5.50
Mineral mix‡	6.25	6.25	6.25	6.25	6.25
Vitamin mix§	1.0	1.0	1.0	1.0	1.0
FO	8	0	0	0	0
AO	0	8	0	0	0
OO	0	0	8	0	0
CO	0	0	0	8	0
SO	2	2	2	2	10

AO, argan oil; CO, coconut oil; FO, fish oil; OO, olive oil; SO, sunflower oil

* Diets were isoenergetic and provided 14.6 MJ/kg of diet.

† Supplied the following amino acids (mg/g diet): arginine, 8.5; cystine, 3; lysine, 17.4; methionine, 7.1; tryptophane, 5.0; glycine, 1.0.

‡ Provided (g/kg diet): $CaHPO_4$ $2H_2O$, 30; KCl, 7; MgO, 3; $MgSO_4$ $7H_2O$, 3.5; Fe_2O_3, 0.2; $FeSO_4$ $7H_2O$, 0.35; $MnSO_4$ H_2O, 0.17; $CuSO_4$ $5H_2O$, 0.03; $ZnSO_4$ $7H_2O$, 0.14; $CoSO_4$ $7H_2O$, 0.0003; stabilized KI, 0.0006.

§ Provided (mg/kg diet): retinyl acetate, 6.93; cholecalciferol, 0.0025; thiamine HCl, 20; riboflavin, 15; *dl*-calcium pantothenate, 70; pyridoxine HCl, 10; meso-inositol, 150; cyanocobalamin, 0.05; all-*rac*-α-tocopheryl acetate, 170; menadione sodium bisulfite, 40; nicotinic acid, 100; choline, 1360; folic acid, 5; *p*-aminobenzoic acid, 50; biotin, 0.3.

The various oil combinations used are presented in Table 1. Concentrate of sardine oil (Radi Holding, Casablanca, Morocco) constituted the ω-3 fatty acid diet (FO diet). Argan oil (Targanine Association, Tamanar, Morocco) constituted the ω-6/ω-9 diet (AO diet). Olive oil (local oil factory, Fes, Morocco) served as the ω-9 diet (OO diet). Coconut oil (Coopération pharmaceutique française, Rhône-Poulenc Rorer, Lyon, France) was used as the saturated fat diet (CO diet). Commercial edible sunflower oil was used as a source of ω-6 fatty acids (SO diet). The diets were thoroughly mixed after the addition of oil combinations and daily amounts were transferred to capped plastic tubes, flushed with nitrogen, and stored at −20°C in the dark. Under these conditions, no oxidation of dietary fat was noticed, as indicated by the absence of thiobarbituric acid-reactive materials. Fresh diets were prepared every 3 d and were fed to the rats daily after uneaten food was discarded. The fatty acid composition of the different diets is presented in Table 2.

Tissue preparation

After 4 wk, rats were anesthetized by diethyl ether and decapitated. Blood was collected immediately on heparin (50 U/mL), and 50 μM butylated hydroxytoluene was added to recovered plasma before freezing at −80°C until further analysis. Thymus glands were removed immediately and thymic lymphocytes were separated on a density gradient as previously described [15]. After washing, cells were suspended at a concentration of 4×10^7cells/mL and incubated for 60 min at 37°C before starting the experiments.

Table 2
Fatty acid composition of experimental diets*

Fatty acid (mol %)	FO diet	AO diet	OO diet	CO diet	SO diet
14:0	8.08 ± 0.28[a]	0.42 ± 0.01[b]	0.37 ± 0.05[b]	29.69 ± 0.65[c]	0.36 ± 0.01[b]
16:0	21.39 ± 0.70[a]	13.49 ± 0.05[b]	11.44 ± 0.17[c]	18.33 ± 0.15[d]	7.45 ± 0.04[e]
16:1*ω-9*	ND	ND	0.40 ± 0.11	ND	ND
16:1*ω-7*	8.85 ± 0.11[a]	ND	0.64 ± 0.15[b]	ND	ND
18:0	4.02 ± 0.14[a]	5.40 ± 0.08[b]	2.55 ± 0.06[c]	5.75 ± 0.13[b]	3.44 ± 0.07[d]
18:1*ω-9*	14.38 ± 0.41[a]	41.19 ± 0.15[b]	62.77 ± 0.58[c]	20.49 ± 0.39[d]	26.49 ± 0.87[e]
18:1*ω-7*	4.12 ± 0.06[a]	ND	0.08 ± 0.08[b]	ND	ND
18:2*ω-6*	16.03 ± 0.35[a]	38.90 ± 0.22[b]	20.25 ± 0.26[c]	25.75 ± 0.50[d]	61.49 ± 0.82[c]
18:3*ω-3*	0.62 ± 0.02	ND	0.72 ± 0.01	ND	ND
20:2*ω-6*	0.22 ± 0.10	0.40 ± 0.02	0.60 ± 0.08	ND	0.23 ± 0.01
20:3*ω-6*	0.19 ± 0.06[a]	0.21 ± 0.01[a]	0.19 ± 0.01[a]	ND	0.55 ± 0.02[b]
20:4*ω-6*	0.55 ± 0.02	ND	ND	ND	ND
20:5*ω-3*	13.34 ± 0.88	ND	ND	ND	ND
22:4*ω-6*	0.95 ± 0.03	ND	ND	ND	ND
22:5*ω-3*	1.25 ± 0.11	ND	ND	ND	ND
22:6*ω-3*	5.93 ± 0.46	ND	ND	ND	ND
SFA	33.49 ± 1.11[a]	19.31 ± 0.11[b]	14.35 ± 0.28[b]	53.76 ± 0.38[c]	11.24 ± 0.08[d]
MUFA	27.44 ± 0.70[a]	41.19 ± 0.15[b]	63.88 ± 0.45[c]	20.49 ± 0.39[d]	26.49 ± 0.87[a]
Σω-6 PUFA	17.93 ± 0.42[a]	39.50 ± 0.22[b]	21.04 ± 0.33[c]	25.75 ± 0.50[d]	62.27 ± 0.82[c]
Σω-3 PUFA	21.14 ± 1.44	ND	0.72 ± 0.01	ND	ND
ω-6/ω-3	0.86 ± 0.05	ND	29.10 ± 0.66	ND	ND
UI[†]	176.87 ± 7.69[a]	120.40 ± 0.31[b]	108.32 ± 0.40[b]	71.99 ± 0.81[c]	151.58 ± 0.81[d]

AO, argan oil; CO, coconut oil; FO, fish oil; MUFA, monosaturated fatty acid; ND, not detected; OO, olive oil; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, sunflower oil; UI, unsaturation index

* Values are means ± SEM ($n = 3$). Different superscript letters denote significant differences ($P < 0.05$). If no superscript appears in a row, the values are not statistically different.

[†] Summed moles per 100 mol multiplied by the number of double bonds.

Fatty acid analysis of phospholipids and triacylglycerols

Total lipids from plasma and thymocytes were extracted according to the method of Bligh and Dyer [16]. Before extraction, triheptadecanoyl glycerol and diheptadecanoyl phosphatidylcholine were added to samples as internal standards. The triacylglycerol (TG) and phospholipid (PL) fractions were separated on silica gel 60 plates (Merck, Darmstadt, Germany) by using an *n*-hexane/diethyl ether/acetic acid (70/30/1, v/v/v) solvent system. Both fractions were scraped off and transmethylated as described previously [17]. The fatty acid methyl esters were analyzed with a Perkin Elmer 5830 gas chromatograph equipped with a capillary column (30 m × 0.32 mm; Supelco, Bellefonte, PA, USA) and flame ionization detection. The column was programmed to increase from 135°C to 160°C at 2°C/min and from 160°C to 205°C at 1.5°C/min; the detection temperature was maintained at 250°C. The vector gas was helium at a pressure of 0.8 lb/in^2 (5520 Pa). Peaks were identified by comparing retention times with those of standard fatty acid methyl esters.

Thymocyte proliferation

Thymocytes were cultured in 96-well culture plates at a cell density of 2.5×10^6 cells/mL in a final volume of 110 μL of RPMI-1640 supplemented with 2 mM glutamine, 0.1 mg/mL of penicillin/streptomycin, and 10% (v/v) fetal calf serum in the presence or absence of concanavalin A (ConA; Sigma-Aldrich, Saint Quentin Fallavier, France). In preliminary experiments, we observed that a concentration of 2.5 μg of ConA/mL produced maximal proliferation (not shown). This ConA concentration was used in subsequent experiments. Cultures were incubated at 37°C in a 95% humidified air/5% CO_2 atmosphere. After 68 h of incubation, cell proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide colorimetric assay (Roche Diagnostics, Meylan, France) as described by Mosmann [18]. The number of viable cells was directly correlated to the difference of absorbance measured at 550 and 690 nm. Cell proliferation was measured as the difference of absorbance between assays with and without ConA.

PLD assay and western blotting

Phospholipase D was assayed based on its transphosphatidylation activity. Thymocytes isolated from rats fed on the different diets were incubated for 1 h at 37°C in the presence of [^{3}H]arachidonic acid (37 kBq/mL, specific activity 7400 GBq/mmol; Amersham Biosciences, Orsay, France) in 0.1% ethanol and then washed three times in RPMI-1640 medium. Arachidonate incorporation yield was consistently in the 54% to 60% range and did not differ significantly across the five dietary groups.

Labeled cells were suspended at a density of 4×10^7 cells/mL and incubated for 20 min at 37°C in the presence of 1% 1-butanol. One microgram of ConA per 10^6 cells was added and cell suspensions were further incubated for 5 min at 37°C. Incubations were terminated by addition of ethanol and acidification of the medium to pH 3 to 4 with 2 M HCl. Lipids were extracted with chloroform/ethanol (6/3, v/v) according to the method of Boukhchache and Lagarde [19] in the presence of 50 μM butylated hydroxytoluene. Phosphatidylbutanol was separated by bidimensional thin-layer chromatography with a chloroform/methanol/28% ammonia (65/35/5.5, v/v/v) solvent system for the first migration and an ethyl acetate/isooctane/acetic acid (9/5/2, v/v/v) solvent system for migration in the second dimension. Spots stained by Coomassie Brilliant Blue R were scraped off and mixed with Picofluor (Perkin Elmer, Courtaboeuf, France), and radioactivity of the spots corresponding to phosphatidylbutanol and phospholipids was determined by liquid scintillation counting. PLD activity was expressed as the percentage of total phospholipid radioactivity incorporated into phosphatidyl butanol.

For western blotting experiments, thymocytes were homogenized in 25 mM Tris-HCl lysis buffer, pH 7.5, containing 150 mM NaCl, 5 mM ethylene diaminetetra acetic acid, and protease inhibitors (protease inhibitor mixture; Sigma). After centrifugation at 900*g* for 10 min, aliquots from the postnuclear supernatant (80 μg of proteins) were precipitated with ice-cold acetone and pellets were dissolved in 20 μL of Laemmli buffer containing 4 M urea and 5 mM ethylene diaminetetra acetic acid. Proteins were denatured by heating at 100°C for 1 min and fractionated with 8% sodium dodecylsulfate polyacrylamide gel electrophoresis including 4 M urea. Blots were probed with PLD1- or PLD2-specific antisera (diluted 1/2000) provided by S. Bourgoin, M.D. (Laval University, Montreal, Quebec, Canada). Horseradish peroxidase-conjugated goat anti-rabbit antibody was used as the secondary antibody. Immunoreactive proteins were visualized with the enhanced chemiluminescence detection system (Amersham Biosciences). After stripping, membranes were reprobed for normalization with an anti–α-tubulin monoclonal antibody (Sigma-Aldrich). Quantifications were performed with a cooled digital CCD camera system (ImageMaster VDS-CL, Amersham Biosciences) and ImageQuant software.

Statistical analysis

Values are presented as means ± SEM of *n* independent experiments. All data were compared by analysis of variance (Statview II for Macintosh, SAS Institute, Cary, NC, USA) followed by protected *t* test. *P* values ≤ 0.05 were considered statistically significant.

Results

After 4 wk of dietary treatment, no significant differences were apparent in final body weight and daily food intake across the five groups (not shown). In contrast, we observed a significant decrease in thymus weight in rats fed the CO diet (0.55 ± 0.02 g, $n = 16$) compared with the four other dietary groups (0.66 ± 0.03 g for the FO group, $n = 16$; 0.65 ± 0.03 g for the AO group, $n = 16$; 0.65 ± 0.02 g for the OO group, $n = 16$; 0.71 ± 0.05 g for the SO group, $n = 6$; CO versus each other group, $P < 0.05$), but this change was not accompanied by a decrease in thymocyte yield (not shown).

Substantial changes in levels and fatty acid composition of plasma TGs were observed depending on the type of oil supplement administered. Rats fed the FO and SO diets had the lowest level of TG (0.42 ± 0.05 and 0.56 ± 0.12 mmol/L, respectively), whereas OO fed animals exhibited the highest level (1.75 ± 0.37 mmol/L), with the AO and CO groups showing intermediate values (1.04 ± 0.14 and 1.00 ± 0.11 mmol/L, respectively). Plasma TGs from OO-fed rats exhibited the highest proportion of 18:1ω-9 (oleic acid, >50%) and the lowest proportion of ω-6 fatty acids, suggesting that 18:1ω-9 enrichment occurred at the expense of ω-6 fatty acids (Table 3). Interestingly, in plasma TG from rats fed the AO supplement, which was rich in 18:1ω-9 and 18:2ω-6 (linoleic acid), the proportion of ω-6 fatty acids was about twice that present in the FO, OO, and CO groups, whereas the proportion of 18:1ω-9 was also increased compared with the FO and SO groups. Unexpectedly, the proportion of ω-6 fatty acids was as low in plasma TG of the OO group as in that of the FO group despite their higher proportion (+17%) in the OO than in the FO diet. This might be due to the high turnover of circulating TGs. In plasma phospholipids, which are metabolically more stable than plasma TGs, proportions of ω-6 fatty acids more closely reflected those of the experimental diets. Moreover, when results are expressed as relative proportions, an increase in a given fatty acid (18:1ω-9 in the OO group) leads to a relative decrease in all the others. In addition, the total mass of plasma TG and of fatty acids they contain has also to be considered, due to the large differences in TG amounts across the various dietary groups. Thus, when expressed as micrograms of fatty acids per milliliter of serum, the amounts of ω-6 fatty acids were 57.49 for FO, 303.98 for AO, 238.02 for OO, 137.62 for CO, and 242.90 for SO, which indicates a higher ω-6 fatty acid content in TG from the OO group than from the FO group. Feeding saturated fat (CO diet) compared with the four other diets resulted in a higher proportion of saturated fatty acids at the expense of polyunsaturated ones. In rats fed the FO diet, we observed a high incorporation of 20:5ω-3 (eicosapentaenoic acid), 22:5ω-3 (docosapentaenoic acid), and 22:6ω-3, which resulted in a drastically lower ω-6/ω-3 ratio compared with those of the four other diets. Among changes in the fatty acid composition of plasma phospholipids observed at the

Table 3
Fatty acid composition of plasma triacylglycerols from rats fed the different diets*

Fatty acid (mol %)	Dietary groups				
	FO	AO	OO	CO	SO
14:0	3.41 ± 0.26[a]	0.90 ± 0.06[b]	1.02 ± 0.06[b]	7.01 ± 0.59[c]	0.82 ± 0.10[b]
16:0	25.99 ± 0.58[a]	22.09 ± 0.55[b,c]	23.16 ± 0.43[b]	30.53 ± 0.45[d]	21.02 ± 1.42[c]
16:1*ω-9*	0.24 ± 0.03[a]	0.38 ± 0.03[b]	0.50 ± 0.05[c]	0.32 ± 0.03[a,b]	0.31 ± 0.08[a,b]
16:1*ω-7*	7.83 ± 0.34[a]	2.67 ± 0.15[b]	4.09 ± 0.26[c]	8.32 ± 0.72[a]	1.40 ± 0.08[b]
18:0	3.12 ± 0.23[a]	2.88 ± 0.12[a]	1.88 ± 0.10[b]	2.34 ± 0.10[c]	2.78 ± 0.15[a,c]
18:1*ω-9*[†]	23.54 ± 0.60[a]	39.67 ± 0.25[c]	54.55 ± 0.41[c]	36.32 ± 1.04[d]	28.12 ± 0.74[e]
18:1*ω-7*					
18:2*ω-6*	12.86 ± 0.37[a]	28.09 ± 0.68[b]	12.88 ± 0.33[a]	13.23 ± 0.74[a]	40.52 ± 2.22[c]
18:3*ω-6*	0.13 ± 0.04[a]	0.38 ± 0.04[b]	0.23 ± 0.02[a,c]	0.25 ± 0.04[c]	0.26 ± 0.09[a,c]
20:2*ω-6*	0.13 ± 0.04[a]	0.38 ± 0.06[b]	0.35 ± 0.06[b]	0.17 ± 0.04[a]	0.46 ± 0.15[b]
20:3*ω-6*	0.20 ± 0.04[a]	0.33 ± 0.04[b]	0.16 ± 0.02[a]	0.21 ± 0.03[a,c]	0.33 ± 0.09[b,c]
20:4*ω-6*	1.60 ± 0.24[a]	1.56 ± 0.08[a]	0.67 ± 0.04[b]	0.83 ± 0.12[b]	2.93 ± 0.34[c]
20:5*ω-3*	11.54 ± 0.50[a]	0.02 ± 0.02[b]	0.01 ± 0.01[b]	ND	ND
22:4*ω-6*	0.16 ± 0.09[a]	0.26 ± 0.05[a,b]	0.10 ± 0.02[a]	0.11 ± 0.04[a]	0.40 ± 0.13[b]
22:5*ω-6*	0.06 ± 0.02[a]	0.28 ± 0.02[b]	0.10 ± 0.01[a]	0.29 ± 0.04[b]	0.59 ± 0.16[c]
22:5*ω-3*	3.40 ± 0.19[a]	0.01 ± 0.01[b]	0.02 ± 0.01[b]	0.02 ± 0.01[b]	ND
22:6*ω-3*	5.61 ± 0.33[a]	0.04 ± 0.01[b]	0.09 ± 0.01[b]	0.06 ± 0.01[b]	0.01 ± 0.01[b]
SFA	32.53 ± 0.57[a]	25.86 ± 0.63[b]	26.06 ± 0.42[b]	39.88 ± 0.72[c]	24.62 ± 1.50[b]
MUFA	31.62 ± 0.65[a]	42.76 ± 0.27[b]	59.20 ± 0.37[c]	44.97 ± 1.04[d]	29.83 ± 0.75[a]
Σω-6 PUFA	15.14 ± 0.51[a]	31.28 ± 0.79[b]	14.48 ± 0.39[a]	15.08 ± 0.93[a]	45.50 ± 2.12[c]
Σω-3 PUFA	20.71 ± 0.61[a]	0.10 ± 0.02[b]	0.26 ± 0.04[b]	0.08 ± 0.02[b]	0.05 ± 0.03[b]
ω-6/ω-3	0.74 ± 0.03[a]	487.71 ± 88.07[b]	88.93 ± 18.57[a,c]	149.48 ± 16.78[c]	351.92 ± 77.43[b]
UI[†]	174.78 ± 3.35[a]	110.97 ± 1.52[b]	91.51 ± 0.80[c]	78.77 ± 1.73[c]	130.04 ± 3.41[e]

AO, argan oil; CO, coconut oil; FO, fish oil; MUFA, monosaturated fatty acid; ND, not detected; OO, olive oil; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, sunflower oil; UI, unsaturation index

* Values are means ± SEM ($n = 16$ for FO, AO, OO, and CO groups; $n = 6$ for SO group). Different superscript letters denote significant differences ($P < 0.05$). If no superscript appears in a row, the values are not statistically different.

[†] Summed moles per 100 mol multiplied by the number of double bonds.

end of the feeding period (Table 4) were the higher content in ω-6 polyunsaturated fatty acids and the higher ω-6/ω-3 ratio in phospholipids from AO-fed versus OO-fed animals. The proportion of monounsaturated fatty acids was markedly higher in thymocyte phospholipids compared with plasma phospholipids mainly due to a higher 18:1ω-9 proportion whatever the diet (Table 5). The ω-6 content of thymocyte phospholipids from AO-fed rats was significantly higher than that from rats fed the FO, OO, and CO diets and quite similar to the level observed in SO rats (38.1 ± 0.8 mol% in AO rats versus 39.1 ± 0.3 mol% in SO rats). Further, the 20:4ω-6 proportion in thymocyte phospholipids was decreased only in FO rats compared with animals from the four other dietary groups. In thymocyte phospholipids from FO rats, the proportion of 20:5ω-3 reached 4.36 mol/100 mol fatty acids but was undetectable in thymocyte phospholipids of rats fed the four other diets. FO feeding also stimulated the incorporation of 22:5ω-3 and 22:6ω-3 at the expense of long-chain ω-6 fatty acids, mainly 20:4ω-6 and 22:4ω-6. As we have previously observed [15], 20:5ω-3 was more efficiently incorporated into thymocytes than into plasma phospholipids as indicated by the 20:5ω-3/22:6ω-3 ratio of 1.65 in plasma versus 5.07 in thymocytes.

Because dietary-induced changes can be lost during lymphocyte culture under standard conditions [20], we first compared the proliferative response of rat thymocytes in medium supplemented with calf serum and in medium supplemented with autologous serum by using five rats in each dietary group. No significant difference in the proliferative response of rat thymocytes to ConA could be detected between cultures with autologous serum and cultures with fetal calf serum, whatever the dietary group considered (not shown). Thus, fetal calf serum was routinely used in subsequent experiments. In the absence of mitogen, thymocyte viability was similar at the end of the feeding period whatever the diet (not shown). In the presence of ConA, thymocytes isolated from SO-fed rats exhibited the highest proliferative response (Fig. 1A). This response was significantly higher than that obtained with thymocytes from rats in the AO, OO, and CO groups ($P < 0.001$) but was similar to that of FO-fed animals (FO group versus AO, OO, and CO groups, $P < 0.02$). The same results were obtained when proliferation was expressed as a stimulation index (not shown). Interestingly, thymocyte proliferation was positively correlated ($P = 0.01$, $r = 0.957$, $n = 5$) to the relative amount of 18:2ω-6 present in their phospholipids (Fig. 1B). PLD activity of ConA-stimulated thymocytes tended to be higher in the CO group than in the other groups, although the difference did not reach statistical significance (Fig. 2A). However a significant negative correlation ($P < 0.05$, $r = 0.880$, $n = 5$) could be established between PLD activity and proliferation (Fig. 2B), suggest-

Table 4
Fatty acid composition of plasma phospholipids from rats fed the different diets*

Fatty acid (mol %)	Dietary groups				
	FO	AO	OO	CO	SO
14:0	0.58 ± 0.03[a]	0.29 ± 0.03[b]	0.27 ± 0.02[b]	1.17 ± 0.10[c]	0.21 ± 0.05[b]
16:0	33.10 ± 0.63[a]	26.75 ± 1.20[b]	25.77 ± 0.51[b]	26.17 ± 0.66[b]	25.55 ± 0.79[b]
16:1*ω-9*	0.12 ± 0.02[a,b]	0.13 ± 0.01[b]	0.18 ± 0.01[c]	0.10 ± 0.01[a,b]	0.02 ± 0.02[d]
16:1*ω-7*	1.89 ± 0.11[a]	0.52 ± 0.05[b]	0.85 ± 0.08[c]	1.31 ± 0.12[d]	0.31 ± 0.02[b]
18:0	18.12 ± 0.50[a]	22.16 ± 0.86[b,c]	20.94 ± 0.35[c]	22.89 ± 0.68[b]	22.24 ± 0.65[b,c]
18:1*ω-9*	4.94 ± 0.29[a,b]	5.91 ± 0.27[c]	9.59 ± 0.25[d]	5.16 ± 0.23[a]	4.12 ± 0.16[b]
18:1*ω-7*	3.63 ± 0.17[a,b]	2.74 ± 0.18[c]	4.21 ± 0.26[a]	3.53 ± 0.24[b]	1.96 ± 0.13[c]
18:2*ω-6*	12.85 ± 0.35[a]	19.23 ± 0.95[b]	16.84 ± 0.40[c]	19.35 ± 0.57[b]	22.14 ± 0.88[d]
18:3*ω-6*	0.10 ± 0.03[a,b]	0.15 ± 0.02[a]	0.15 ± 0.02[a]	0.16 ± 0.03[a]	0.02 ± 0.02[b]
20:2*ω-6*	0.04 ± 0.02[a]	0.35 ± 0.04[b]	0.31 ± 0.08[b,c]	0.19 ± 0.04[a,c]	0.45 ± 0.10[b]
20:3*ω-6*	0.67 ± 0.14[a]	0.77 ± 0.08[a,b]	0.89 ± 0.06[b]	1.18 ± 0.07[c]	0.55 ± 0.04[a]
20:4*ω-6*	9.62 ± 0.36[a]	17.79 ± 0.70[b,c]	16.84 ± 0.46[b]	16.27 ± 0.65[b]	19.44 ± 0.70[c]
20:5*ω-3*	7.53 ± 0.28[a]	0.09 ± 0.02[b]	0.14 ± 0.02[b]	0.08 ± 0.02[b]	ND
22:4*ω-6*	0.62 ± 0.20	0.66 ± 0.16	0.49 ± 0.14	0.25 ± 0.09	0.44 ± 0.03
22:5*ω-6*	0.07 ± 0.04[a]	1.63 ± 0.13[b]	0.79 ± 0.06[c]	1.48 ± 0.11[b]	1.46 ± 0.22[b]
22:5*ω-3*	1.91 ± 0.11[a]	0.12 ± 0.03[b]	0.21 ± 0.02[b]	0.10 ± 0.02[b]	0.16 ± 0.04[b]
22:6*ω-3*	4.56 ± 0.22[a]	1.15 ± 0.18[b]	2.03 ± 0.08[c]	1.31 ± 0.07[b]	0.93 ± 0.08[b]
SFA	51.81 ± 0.63[a]	49.21 ± 1.76[a,b]	46.98 ± 0.54[b]	50.22 ± 1.21[a]	47.99 ± 0.96[a,b]
MUFA	10.61 ± 0.35[a]	9.36 ± 0.33[b]	14.88 ± 0.29[c]	10.14 ± 0.36[a,b]	6.41 ± 0.11[d]
Σω-6 PUFA	23.58 ± 0.44[a]	40.09 ± 1.44[b]	35.76 ± 0.50[c]	38.16 ± 0.97[b,c]	44.50 ± 0.87[d]
Σω-3 PUFA	14.01 ± 0.44[a]	1.35 ± 0.18[b]	2.38 ± 0.07[c]	1.48 ± 0.08[b]	1.10 ± 0.10[b]
ω-6/ω-3	1.71 ± 0.07[a]	33.36 ± 2.61[b]	15.23 ± 0.43[c]	26.51 ± 1.16[d]	42.14 ± 3.21[e]
UI[†]	153.40 ± 2.56[a]	139.64 ± 4.84[b,c]	137.82 ± 2.09[b,c]	131.16 ± 5.58[c]	146.53 ± 3.32[a,b]

AO, argan oil; CO, coconut oil; FO, fish oil; MUFA, monosaturated fatty acid; ND, not detected; OO, olive oil; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, sunflower oil; UI, unsaturation index.

* Values are means ± SEM ($n = 14$ for FO and AO groups; $n = 16$ for OO group; $n = 15$ for CO group; $n = 6$ for SO group). Different superscript letters denote significant differences ($P < 0.05$). If no superscript appears in a row, the values are not statistically different.

[†] Summed moles per 100 mol multiplied by the number of double bonds.

ing that high PLD activity might impair the proliferative response of rat thymocytes to mitogens. The highest expression of PLD1 protein was observed in thymocytes from the AO- and OO-fed rats, whereas the lowest PLD2 protein expression was observed in thymocytes from FO-fed animals (Fig. 3A,B). It is noteworthy that the pattern of PLD2 protein expression roughly reflected that of PLD activity measured in intact cells ($P = 0.08$, not significant), with the exception of thymocytes from SO-fed rats, which exhibited low PLD activity and high PLD2 expression.

Discussion

Due to its hypolipemic and antioxidant properties, argan oil is being recognized as a valuable nutritional supply for the prevention of cardiovascular diseases [3,5]. Because dietary fatty acids also affect the regulation and function of the immune system [7,8], we sought to determine the effects of argan oil consumption on immune cells in the rat.

A short feeding period of 4 wk was sufficient to influence plasma TG levels. The TG-lowering effect of the FO diet that we observed in the present study has been consistently reported in human and animal studies [21,22]. Although OO-enriched diets do not seem to affect triglyceridemia in humans [23], several studies have reported increased plasma TG levels after OO feeding in rats [24,25], as we observed in the present study. Interestingly, the AO diet led to TG values that were intermediate between those induced by the FO and OO diets. At the end of the feeding period, large variations were observed in fatty acid composition of plasma TGs and phospholipids, which grossly reflected that of the experimental diets. In plasma phospholipids, the proportion of saturated fatty acids was quite similar whatever the diet. Thus, the high content of saturated fatty acids available in the CO diet did not increase uptake in plasma phospholipids. These results are in accordance with those reported in most dietary studies and are due to the fact that saturated fatty acids at the sn-1 position of the glycerol backbone of phospholipids cannot easily undergo replacement by unsaturated ones [26]. The monoenoic acid proportion was significantly decreased in plasma phospholipids from rats fed the SO diet compared with rats fed the four other diets. This was very likely due to competition between monoenoic and ω-6 fatty acids for esterification at the sn-2 position of glycerol [27]. An alternative explanation might be that the high supply of linoleic acid in the SO diet inhibited Δ9-desaturase activity [28]. However, endogenous synthesis of monoenoic acids is not very likely to occur due to their large supply in the diet. Interestingly, argan oil,

Table 5
Fatty acid composition of thymocyte phospholipids from rats fed different diets*

Fatty acid (mol %)	Dietary group				
	FO	AO	OO	CO	SO
14:0	0.86 ± 0.07[a]	0.69 ± 0.05[a]	0.67 ± 0.05[a]	1.24 ± 0.11[b]	0.64 ± 0.06[a]
16:0	26.34 ± 0.34	26.57 ± 0.77	26.91 ± 0.45	26.50 ± 0.69	27.42 ± 0.16
16:1(*ω-9*)	0.61 ± 0.02[a]	0.45 ± 0.02[b]	0.63 ± 0.03[a]	0.48 ± 0.03[b]	0.42 ± 0.01[b]
16:1(*ω-7*)	1.89 ± 0.05[a]	0.83 ± 0.04[b]	1.03 ± 0.06[c]	1.20 ± 0.07[d]	0.82 ± 0.03[b,c]
18:0	17.20 ± 0.24[a]	18.66 ± 0.31[b]	17.70 ± 0.24[a]	18.68 ± 0.33[b]	17.81 ± 0.24[a,b]
18:1(*ω-9*)	10.89 ± 0.39[a]	9.04 ± 0.26[b]	11.29 ± 0.28[a]	8.25 ± 0.30[b]	8.68 ± 0.16[b]
18:1(*ω-7*)	8.17 ± 0.24[a]	5.60 ± 0.11[b]	6.55 ± 0.22[c]	7.16 ± 0.26[d]	5.04 ± 0.17[b]
18:2(*ω-6*)	8.52 ± 0.28[a]	7.09 ± 0.16[b]	5.78 ± 0.12[c]	5.80 ± 0.15[c]	8.89 ± 0.28[a]
18:3(*ω-6*)	0.16 ± 0.04	0.17 ± 0.04	0.18 ± 0.03	0.19 ± 0.04	ND
20:2(*ω-6*)	0.74 ± 0.04[a]	1.91 ± 0.08[b]	1.09 ± 0.08[c]	1.30 ± 0.19[c]	2.71 ± 0.15[d]
20:3(*ω-6*)	1.46 ± 0.02[a]	1.10 ± 0.03[b,c]	1.09 ± 0.05[b,c]	1.17 ± 0.05[b,d]	0.97 ± 0.08[c]
20:4(*ω-6*)	15.29 ± 0.31[a]	24.07 ± 0.58[b]	24.10 ± 0.35[b]	24.74 ± 0.56[b]	24.24 ± 0.46[b]
20:5(*ω-3*)	4.36 ± 0.16[a]	ND	ND	ND	ND
22:4(*ω-6*)	1.07 ± 0.25[a]	2.97 ± 0.30[b]	2.58 ± 0.20[b,c]	2.53 ± 0.19[b,c]	1.94 ± 0.10[a,c]
22:5(*ω-6*)	0.13 ± 0.06[a]	0.76 ± 0.08[b]	0.34 ± 0.06[c]	0.74 ± 0.08[b]	0.32 ± 0.09[a,c]
22:5(*ω-3*)	1.47 ± 0.09	ND	ND	ND	ND
22:6(*ω-3*)	0.86 ± 0.04[a]	0.08 ± 0.03[b]	0.07 ± 0.03[b]	0.02 ± 0.02[b]	0.10 ± 0.05[b]
SFA	44.40 ± 0.32[a]	45.93 ± 0.82[a,b]	45.28 ± 0.31[a,b]	46.42 ± 0.91[b]	45.87 ± 0.18[b]
MUFA	21.56 ± 0.34[a]	15.92 ± 0.23[b]	19.50 ± 0.23[c]	17.09 ± 0.41[d]	14.96 ± 0.15[b]
Σ*ω*-6 PUFA	27.36 ± 0.35[a]	38.07 ± 0.80[b]	35.15 ± 0.50[c]	36.46 ± 0.64[c]	39.07 ± 0.25[b]
Σ*ω*-3 PUFA	6.68 ± 0.18[a]	0.08 ± 0.03[b]	0.07 ± 0.03[b]	0.02 ± 0.02[b]	0.10 ± 0.05[b]
ω-6/*ω*-3	4.15 ± 0.15[a]	214.65 ± 19.55[b]	132.74 ± 10.98[c]	ND	245.28 ± 47.97[b]
UI[†]	145.26 ± 1.39	149.83 ± 3.04	145.87 ± 1.53	148.27 ± 2.77	148.00 ± 1.19

AO, argan oil; CO, coconut oil; FO, fish oil; MUFA, monosaturated fatty acid; ND, not detected; OO, olive oil; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; SO, sunflower oil; UI, unsaturation index

* Values are means ± SEM ($n = 15$ for FO, AO, OO, and CO groups; $n = 5$ for SO group). Different superscript letters denote significant differences ($P < 0.05$). If no superscript appears in a row, the values are not statistically different.

[†] Summed moles per 100 mol multiplied by the number of double bonds.

which contains less 18:2*ω*-6 than does sunflower oil, did not have the same negative effect on monoenoic content.

Although thymocytes are relatively resistant to changes induced by dietary fatty acids [29], we observed significant modifications in the fatty acid pattern of their phospholipids at the end of the feeding period. The main variations between AO-fed and OO-fed animals concerned the proportions of monoenoic and *ω*-6 fatty acids. Thymocyte phospholipids from AO-fed rats had significantly lower levels of 16:1*ω*-9, 16:1*ω*-7, 18:1*ω*-9, and 18:1*ω*-7 than those from OO-fed rats. Conversely, they exhibited higher levels of 18:2*ω*-6, 20:2*ω*-6, and 22:5*ω*-6. It is noteworthy that, although the 18:2*ω*-6 content of phospholipids varied across groups, with the largest amount being observed in SO- and FO-fed rats and the lowest in OO- and CO-fed animals, the proportion of 20:4*ω*-6 remained relatively constant in all but the FO group. Feeding long-chain *ω*-3 fatty acids drastically decreased the proportion of 20:4*ω*-6 despite a high 18:2*ω*-6 proportion, likely due to its replacement by *ω*-3 polyunsaturated fatty acid through the acylation/deacylation process and to inhibition of Δ6-desaturase and the elongase system by *ω*-3 fatty acids.

Feeding the AO diet, which contained equivalent large amounts of oleic and linoleic acids, or the OO diet significantly decreased proliferation of thymocytes compared with feeding the SO and FO diets, whereas thymocytes from the CO group exhibited the lowest proliferative response. Differences in the production of immunosuppressive eicosanoids could not account for these results because the phospholipid proportion of 20:4*ω*-6 was similar in thymocytes that exhibited the lowest proliferative response (AO, OO, and CO groups) and in thymocytes from the SO group, which exhibited the highest proliferative response. The present results are in good agreement with those of Jeffery et al. [30] who reported that feeding OO or a high oleic SO decreased the mitogen-stimulated spleen lymphocyte proliferation compared with feeding SO or a low-fat diet. Similarly, Sierra et al. [31] reported that feeding mice a high oleic SO decreased spleen lymphocyte proliferation compared with feeding rice bran oil, which is rich in linoleic acid. It is noteworthy that, in the present study, a significant positive linear relation was found between thymocyte proliferation and the relative amount of 18:2*ω*-6 present in thymocyte phospholipids. Thymocyte proliferation also tended to be negatively correlated to the 18:1*ω*-9/18:2*ω*-6 ratio of phospholipids ($P = 0.1$, not shown). Similar relations have been reported by Jeffery et al. [32] who observed a negative correlation between proliferation of rat splenic lymphocytes and the ratio of oleic to linoleic acid in the diets. In contrast, Moussa et al. [33] reported the opposite results in rats fed high- and low-fat diets containing coconut or soybean oil.

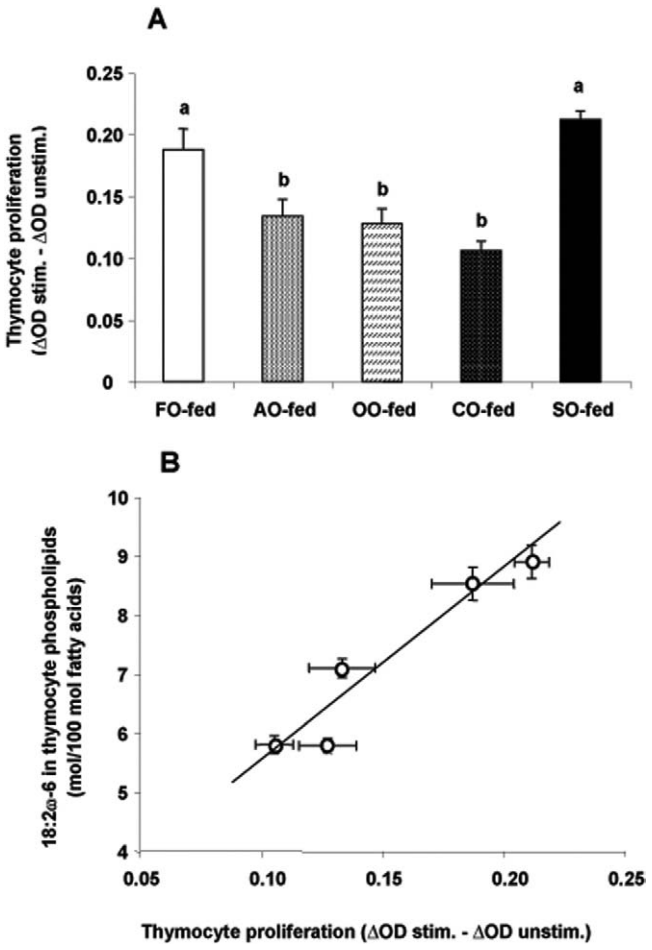

Fig. 1. Effects of dietary lipid manipulation on the proliferation of concanavalin A–stimulated thymocytes. (A) At the end of the feeding period, thymocytes were isolated as described in MATERIALS AND METHODS, suspended in complete culture medium containing 10% (v/v) fetal calf serum at a density of 2.5×10^6 cells/mL, and cultured for 68 h in the presence of 2.5 μg of concanavalin A/mL. Proliferation was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide proliferation kit according to the manufacturer's recommendations. Data are means ± SEM of 14 (FO, AO, OO, and CO groups) or 6 (SO group) separate experiments with each performed with six replicates. Different letters denote significant differences ($P < 0.05$). (B) Positive linear relation between concanavalin A–induced proliferation and relative amount of 18:2ω-6 present in thymocyte phospholipids (data from A and Table 5; $P = 0.01$, $r = 0.957$, $n = 5$). AO, argan oil; CO, coconut oil; ΔOD stim., ??; ΔOD unstim., ??; FO, fish oil; OO, olive oil; SO, sunflower oil.

Changes in the fatty acid composition of lipid membranes induced by dietary lipids modify the biophysical properties of cell membranes, which may in turn influence the mobility and function of membrane proteins [7,8]. The different diets used in the present study induced a complex modification pattern of membrane-bound PLD. When examined at a gross level, the most prominent feature was the significant inverse relation between PLD activity of intact thymocytes and their proliferation in response to ConA activation. These results support further our current hypothesis that PLD might be antiproliferative in lymphoid cells [9]. This hypothesis is also supported by several studies conducted in B cells [14,34]. We then tried to delineate which PLD isoform was preferentially affected by dietary fatty acid manipulations. We previously reported that incorporation of docosahexaenoic acid in cell phospholipids selectively stimulates the PLD1 activity of human lymphocytes [9]. In contrast, Kim et al. [35] examined recombinant human PLD1 (hPLD1) and hPLD2 and observed a selective activation of PLD2 by unsaturated fatty acids brought to the reaction medium as sodium salts. At the end of the feeding period, we observed that PLD1 protein was significantly less abundant in thymocytes from FO-fed rats than in thymocytes from the AO- and OO-fed rats,

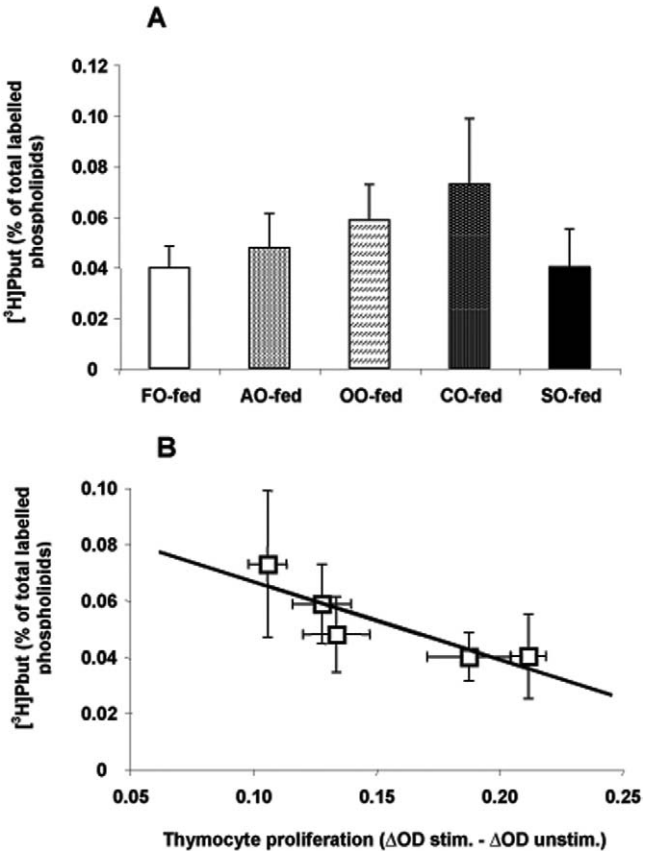

Fig. 2. Effects of the different diets on phospholipase D activity of concanavalin A–stimulated thymocytes. At the end of the feeding period, thymocytes were isolated as described in MATERIALS AND METHODS, suspended at a cell density of 40×10^6 cells/mL, and radiolabeled with ^{3}[H]arachidonic acid. Radiolabeled thymocytes were incubated for 20 min in the presence of 1-butanol (1% final concentration) and then stimulated or not with 1 μg of concanavalin A per 10^6 cells for 5 min. (A) Phospholipase D activity was measured as described in MATERIALS AND METHODS. Results are expressed as percentages of total phospholipid radioactivity incorporated into Pbut and are means ± SEM of 14 (FO, AO, and CO groups), 15 (OO group), or 6 (SO group) separate experiments. (B) Inverse linear relation between phospholipase D activity and concanavalin A–induced proliferation (data from Fig. 1A and 2A; $P = 0.04$, $r = 0.880$, $n = 5$). AO, argan oil; CO, coconut oil; ΔOD stim., OD 550nm–OD690nm for ConA-stimulated thymocytes; ΔOD unstim., OD 550nm–OD690nm for unstimulated thymocytes; FO, fish oil; OO, olive oil; Pbut, phosphatidyl butanol; SO, sunflower oil.

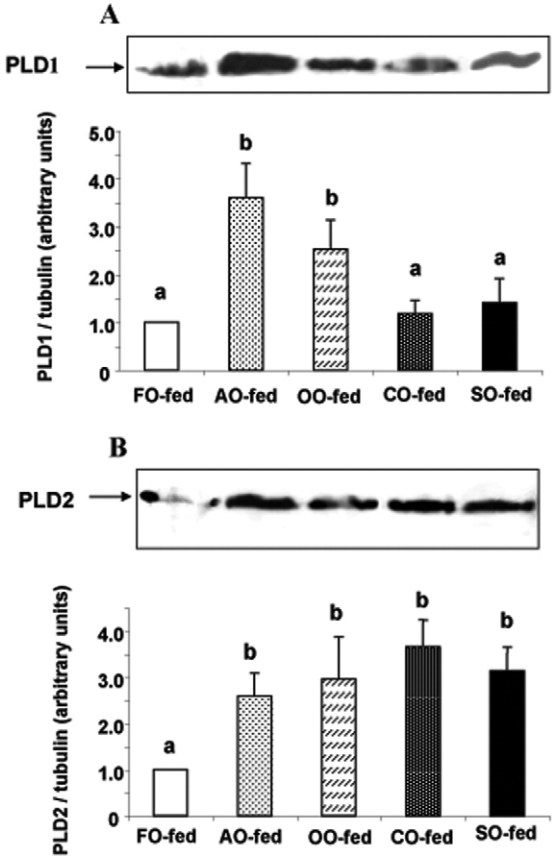

Fig. 3. Effects of different diets on PLD1 and PLD2 expression. At the end of the feeding period, thymocyte extracts were prepared as described in MATERIALS AND METHODS and fractionated by sodium dodecylsulfate polyacrylamide gel electrophoresis. Membranes were probed with (A) an anti-PLD1 antiserum and (B) an anti-PLD2 antiserum. Diagrams show the videodensitometric quantitations of PLD1 (A) and PLD2 (B) proteins. Blots were reprobed for tubulin (not shown) and PLD amounts were normalized by tubulin. Means ± SEM of four separate experiments are shown. Different letters denote significant differences ($P < 0.05$). AO, argan oil; CO, coconut oil; FO, fish oil; OO, olive oil; PLD1, phospholipase D1 protein; PLD2, phospholipase D2 protein; SO, sunflower oil.

whereas PLD2 protein was expressed at the lowest level in FO-fed rats compared with rats in the other groups. These results indicated that PLD activity measured in intact thymocyte is better correlated to PLD2 than to PLD1 protein level.

In conclusion, the present findings show that the effects of AO on immune cells are very similar to those of OO. Although the immunosuppressive effects of OO consumption seem to be greater in animals than in humans, some clinical studies have reported beneficial anti-inflammatory effects in some pathologic conditions such as autoimmune disorders [36]. Similarly to OO, AO contains a variety of antioxidant molecules such as plant sterols, polyphenols, and tocopherols, which may have, in addition to the specific effects of fatty acid composition, beneficial effects against inflammatory disorders [5]. Thus, AO can be used as a balanced dietary supply to decrease cardiovascular risk factors without marked adverse effects on immune cell function.

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