

Relationship between the fatty acid composition of rat lymphocytes and immune functions

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The effects of dietary lipids on the fatty acid composition, activation and proliferation of lymphocytes were investigated. Weanling male Wistar rats were fed for 8 weeks on one of two low-fat diets which contained 50 g lipid/kg, or one of two high-fat diets containing 200 g lipid/kg, from either coconut oil or soyabean oil. The fatty acid composition of phospholipids from splenocyte membranes was affected by dietary lipid manipulation, and these differences influenced lymphocyte functions. Increased levels of linoleic acid in spleen lymphocytes correlated negatively with interleukin-2 receptor α -chain expression determined either by measuring the mean fluorescence or by the proportion of cells staining positive for CD25, and with the cell proliferation index. However, we found a positive correlation between interleukin-2 receptor α -chain expression determined by measuring the mean fluorescence and the cell proliferation index with the oleic acid concentration of spleen lymphocytes. Since phospholipid hydrolysis occurs early in lymphocyte activation, immunosuppressive effects induced by polyunsaturated fatty acids, described in the literature, could be due to an increase of linoleic acid or a decrease of oleic acid affecting many components of plasma-membrane-associated events involved in lymphocyte activation.

Lymphocyte proliferation: Oleic acid: Linoleic acid: Interleukin-2 receptor

The lipid composition and architecture of the cell membrane play important roles in many aspects of cellular physiology. The fatty acid composition of dietary lipids induces modifications of the cell membranes (Holman, 1968). This phenomenon also concerns lymphocyte membranes, structural modifications of which might influence lymphocyte functions.

In vitro investigations of the effects of fatty acids have shown that polyunsaturated fatty acids (PUFA) inhibit mitogen-stimulated proliferation of lymphocytes (Tsang *et al.* 1977; Calder *et al.* 1991, 1992, 1994; Calder & Newsholme, 1992*a,b*; Soyland *et al.* 1993). Moreover, unsaturated fatty acids have also been shown to suppress the production of cytokines by mitogen-stimulated rat and human lymphocytes (Calder & Newsholme, 1992*b*). Studies of the effects of fatty acids on the expression of receptors for interleukin-2 (IL-2) and transferrin by lymphocytes have shown that PUFA decrease the expression of the transferrin receptors by concanavalin A (ConA)-stimulated rat lymph-node cells (Calder & Newsholme, 1992*b*).

Dietary studies in which animals have been fed on PUFA-containing oils have yielded contradictory findings. Diets

rich in linoleic acid have been shown to have no effect (Lockniskar *et al.* 1983), to enhance (Ossman *et al.* 1980) or to inhibit (Levy *et al.* 1983; Erickson *et al.* 1983; Meydani *et al.* 1991; Calder *et al.* 1995) lymphocyte proliferation. More recently, several studies (Berger *et al.* 1993; Yaqoob *et al.* 1994; Sanderson *et al.* 1995; Jeffery *et al.* 1996) have reported that feeding rats on diets containing very large amounts of olive oil significantly decreases spleen lymphocyte proliferation and natural killer-cell activity. Detailed mechanisms responsible for these functional changes remain largely undetermined.

Membrane phospholipid degradation occurs early in lymphocyte activation. Mitogens like ConA bind to cell membrane glycoproteins, including the T-cell receptor-CD3 complex (Weiss *et al.* 1987). Mitogens acting on the T-cell receptor-CD3 complex lead to stimulation of phospholipases and to formation of second messengers which act to stimulate a cascade of reactions involving transmembrane proteins. This, in turn, leads to activation of distinct transcriptional regulators inducing IL-2 gene and IL-2 receptor α -chain gene expression. Autocrine usage of IL-2 on its receptor induces lymphocyte proliferation. Consequently,

Abbreviations: ConA, concanavalin A; HF, high fat; IL-2, interleukin-2; LF, low fat; PUFA, polyunsaturated fatty acids.

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modifications in dietary fatty acid intake might lead to a modification in phospholipid fatty acid composition, which could determine, after cell stimulation, alterations in this cascade of events. Thus, we decided to study how modifications in the fatty acid composition of spleen lymphocyte phospholipids, induced by dietary lipid manipulations, could influence the immune response. We studied the expression of the IL-2 receptor α -chain which reflects lymphocyte activation, and lymphocyte proliferation. We examined the effect on these lymphocyte functions of feeding weanling rats on different dietary lipids for 8 weeks.

Materials and methods

Rats and diets

The studies were approved by the French Ministry of Agriculture, Fisheries and Nutrition. Weanling male Wistar rats (R. Janvier, Le Genest sur Isle, France), weighing 64 (SE 0.96) g, n 28, were housed in individual cages at 27° as soon as they arrived in the laboratory, and were given free access to food and water. The rats were fed on four different diets (Institut National de Recherche Agronomique, Jouy-en-Josas, France) for 8 weeks (n 7 per diet). Two low-fat (LF) diets contained 50 g lipid/kg from either coconut oil or soyabean oil and two high-fat (HF) diets contained 200 g lipid/kg from either coconut oil or soyabean oil. The compositions of the diets are shown in Table 1. The fatty acid compositions of the diets are shown in Table 2.

The coconut-oil diets contained a high proportion of medium-chain saturated fatty acids, particularly lauric acid (12:0). Most of the other fatty acids in these diets were long-chain saturated fatty acids such as myristic (14:0) and palmitic acids (16:0), although oleic (18:1 n -9) and linoleic (18:2 n -6) acids were present. The soyabean-oil diets contained approximately (g/100 g total fatty acids): 55 linoleic acid, 22 oleic acid and 8 α -linolenic acid. Linoleic acid was the main PUFA of the diets. The minimum amounts of linoleic acid were: 0.87 % energy in

Table 1. Compositions (g/kg) of high-fat and low-fat experimental diets

Ingredients	High fat	Low fat
Maize starch	345	442
Cellulose	19	19
Sucrose	180	231.3
Casein	211	211
DL-Methionine	1.3	1.3
Lipids*	201	52.7
Mineral mix†	32.7	32.7
Vitamin mix‡	10	10

* The diet contained either coconut oil or soyabean oil.

† Provided (mg/kg diet): CaHPO₄·2H₂O 380, K₂HPO₄ 240, CaCO₃ 181, NaCl 70, MgO 20, MgSO₄·7H₂O 90, FeSO₄·7H₂O 7, ZnSO₄·H₂O 5, MnSO₄·H₂O 5, CuSO₄·5H₂O 1, NaF 1, Al₂(SO₄)₃·K₂SO₄·24H₂O 0.2, KCl 0.08, CoCO₃ 0.08, Na₂SeO₃·5H₂O 0.01.

‡ Provided (/kg diet): α -tocopherol 4.55 g, L-ascorbic acid 0.45 g, choline chloride 75 g, D-calcium pantothenate 3 g, inositol 5 g, menadione 2.25 g, niacin 4.5 g, para-aminobenzoic acid 5 g, pyridoxine HCl 1 g, riboflavin 1 g, thiamin HCl 1 g, retinyl acetate 310 mg, ergocalciferol 2.5 mg, biotin 20 mg, pteroylmonoglutamic acid 90 mg, cyanocobalamin 1.35 mg.

Table 2. Fatty acid compositions (g/100 g total fatty acids) of the high-fat (HF) and low-fat (LF) diets containing coconut oil (C) or soyabean oil (S)*

Fatty acid	Dietary group			
	CLF	CHF	SLF	SHF
12:0	45.9	47.2	–	–
14:0	18.8	19.1	–	–
16:0	11.6	10.5	10.8	10.8
18:0	3.8	3.7	3.9	3.8
18:1 n -9	11.6	11.2	22.3	22.0
18:2 n -6	7.4	7.5	55.2	55.2
18:3 n -3	<1.0	<1.0	7.6	8.2

* The two low-fat diets contained 50 g lipid/kg and the two high-fat diets contained 200 g lipid/kg.

the coconut LF diet, 2.86 % energy in the coconut HF diet and 6.84 % energy in the soyabean LF diet; the maximum amount was 21.6 % energy in the soyabean HF diet.

Spleen lymphocyte preparation and culture

The rats were killed by exsanguination under light diethyl ether anaesthesia via the abdominal aorta after laparotomy and each spleen was removed and placed in 5 ml RPMI 1640 culture medium (Eurobio, Paris, France). The spleens were dissected and homogenized in this medium. Mononuclear cells were recovered after Ficol-Hypaque (Eurobio) density gradient centrifugation (d 1.077) at 18 000 g for 20 min at 20°. Cells were washed and resuspended in RPMI.

The proportion of viable cells (95 %) was determined by Trypan blue dye exclusion. The cells were cultured in ninety-six-well micro-titre plates at 37° in an air-CO₂ (19:1 v/v) atmosphere at a density of 2×10^5 cells/well in RPMI supplemented with 2 mmol/l glutamine and antibiotics (penicillin, streptomycin, neomycin) and, to stimulate lymphocytes, 5 μ g/ml ConA (Sigma, Saint Quentin Fallavier, France), the optimal concentration of ConA having been determined previously. The culture medium was also supplemented with autologous serum (25 ml/l).

Measurement of spleen lymphocyte proliferation

After 48 h lymphocyte culture, Mossmann's colorimetric assay with tetrazolium salts (Mossmann, 1983) was used for measurement of viable cell number. The absorbance of each sample and control (ConA-free medium) was read on a spectrophotometer at 565 nm in triplicate, and the proliferation index was defined as: (absorbance sample – absorbance blank)/(absorbance blank).

Lipid analysis

Lymphocytes were prepared as described earlier. Lipids were extracted with a chloroform-methanol (2:1, v/v) mixture (Folch *et al.* 1957). Total phospholipids were separated by TLC on silica gel 60 plates (E. Merck, Darmstadt, Germany) using a diethyl ether-acetone mixture (90:30, v/v) as the mobile phase. GC was used to analyse total phospholipid fatty acids as previously described

(Garcia *et al.* 1986). Results are expressed as g/100 g total fatty acids.

Phenotyping of lymphocyte populations

Fluorescence-activated cell sorter (FACS) analysis was used to measure the presence of various markers on the surface of lymphocytes. Cytofluorimetric analysis was performed through a FACSCAN (Becton-Dickinson, San Jose, CA, USA) equipped with a single 488 nm Ar laser. Monoclonal antibodies were supplied by Serotec (Oxford, UK) and tricolour-streptavidin by Caltag-Tébu (Le Perray en Yvelines, France). Approximately 2×10^5 cells (resuspended in PBS) were incubated for 30 min at 4° with monoclonal antibodies: anti-CD4 (W3/25 biotin), anti-CD8 (MRC 0X 8 biotin) major histocompatibility complex class II as B lymphocyte marker (MRC 0X 6 phycoerythrin) and ED1 fluorescein isothiocyanate as monocyte marker. The cells were then washed twice with PBS and incubated for 30 min at 4° with tricolour-streptavidin for detection of anti-CD4 and anti-CD8 biotinylated monoclonal antibodies. After washing twice with PBS, the cells were suspended in formaldehyde in PBS (20 ml/l) and examined through a cytofluorometer. Viable cells (10^4), were collected and gated using forward and side light scatters. The distribution of fluorescently-labelled cells was computed using LYSYS II software (Becton-Dickinson).

Analysis of interleukin-2 receptor α -chain (CD25) expression

Flow cytometry was used to measure the expression of the IL-2 receptor α -chain. Lymphocytes were cultured as described earlier. After 48 h, the cells were removed and washed twice in PBS. Then the cells were incubated for 30 min at 4° with monoclonal antibody to the α -chain of the IL-2 receptor, anti-CD25 (MRC 0X 39 phycoerythrin).

After washing twice with PBS, the cells were suspended in formaldehyde in PBS (20 ml/l) and examined using cytometric fluorescence-activated cell sorter analysis.

Statistics

All statistical analyses were computed with an Apple Macintosh-compatible statistical package Systat 5.1 (Systat Inc., Evanston, IL, USA). Two-way ANOVA, using fat source and fat level as the independent variables, was used to investigate significant differences of the summary variables. The Pearson correlation coefficient was used to test the relationships between the immunological variables studied and amounts of linoleic or oleic acid in lymphocyte membranes. The amounts of fatty acids in lymphocyte membranes (g/100 g total fatty acids) are means for four rats per diet and those for IL-2 receptor α -chain expression or proliferation index are means for five rats per diet.

Results

Fatty acid composition of spleen lymphocytes (Table 3)

Levels of monounsaturated fatty acids (16:1n-7 and 18:1n-9) and linoleic acid in spleen lymphocytes were significantly modified by the fat source ($P=0.016$, 0.005 and 0.001 respectively) and the fat level ($P=0.001$, 0.023 and 0.001 respectively). Levels of monounsaturated fatty acids decreased with the HF diets compared with the LF diets and also decreased with the soyabean-oil diets compared with the coconut-oil diets. Docosapentaenoic acids (22:5n-6 and 22:5n-3) were only influenced by fat source; 22:5n-6 was significantly lower ($P=0.017$) in the soyabean-oil diet group than in the coconut-oil diet group while, in contrast, 22:5n-3 was significantly greater in the soyabean oil group ($P=0.012$).

Table 3. Fatty acid compositions (g/100 g total fatty acids) of the phospholipid fractions of spleen lymphocytes from rats fed on high-fat (HF) and low-fat (LF) diets containing coconut oil (C) or soyabean oil (S)*
(Mean values with their standard errors)

Fatty acid	Dietary group								Statistical significance of effect of: ($P=$)	
	CLF (n 3)		CHF (n 4)		SLF (n 4)		SHF (n 5)		Fat source	Fat level
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM		
16:0	25.34	2.23	23.04	2.10	25.48	1.73	22.04	3.60	NS	NS
18:0	17.75	1.09	20.17	1.41	18.09	1.22	20.10	1.83	NS	NS
16:1n-7	1.12	0.14	0.58	0.05	0.80	0.13	0.33	0.01	0.016	0.001
18:1n-9	9.61	0.37	7.74	0.70	7.31	0.20	6.63	0.30	0.005	0.023
18:2n-6	5.71	0.55	9.35	0.39	10.51	0.92	13.90	0.96	<0.001	0.001
20:3n-6	1.26	0.14	2.02	0.40	1.46	0.05	1.36	0.09	NS	NS
20:4n-6	25.46	0.60	24.70	0.94	23.15	0.55	23.85	1.74	NS	NS
22:4n-6	3.07	0.16	3.33	0.29	3.02	0.18	2.91	0.60	NS	NS
22:5n-6	0.92	0.15	0.61	0.25	0.35	0.06	0.22	0.71	0.017	NS
22:5n-3	0.62	0.12	0.99	0.04	1.24	0.21	1.30	0.17	0.012	NS
22:6n-3	1.47	0.32	1.29	0.25	1.92	0.56	1.48	0.22	NS	NS
20:3n-9	1.52	0.19	1.38	0.26	1.53	0.34	2.33	0.20	NS	NS

* For details of diets and procedures, see Table 1 and p. 328.

Table 4. Interleukin-2 receptor α -chain (CD25) expression and cell proliferation of spleen lymphocytes from rats fed on high-fat (HF) and low-fat (LF) diets containing coconut oil (C) or soyabean oil (S)*
(Mean values with their standard errors)

Dietary group . . .	CLF (n 5)		CHF (n 4)		SLF (n 4)		SHF (n 5)		Statistical significance of effect of: (P=)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Fat source	Fat level
CD25 expression										
Positive cells (%)	57.40	4.81	55.50	3.97	44.25	8.46	37.00	8.70	0.040	NS
Fluorescence	67.40	3.59	61.70	3.64	53.50	6.98	47.40	6.42	0.021	NS
Proliferation index	1.58	0.12	1.03	0.18	0.93	0.14	0.54	0.13	0.001	0.005

*For details of diets and procedures, see Table 1 and p. 328.

Lymphocyte subpopulations

Fluorescence-activated cell sorter analysis using monoclonal antibodies directed against CD4, CD8, a B-cell marker or a monocyte marker showed that lymphocyte preparations from spleens consisted of approximately 60% T-cells (33% of cells were CD4⁺ and 26% were CD8⁺), 25% B-cells and 3% monocytes. There was no significant effect of dietary lipid manipulation on lymphocyte subpopulations in the spleen.

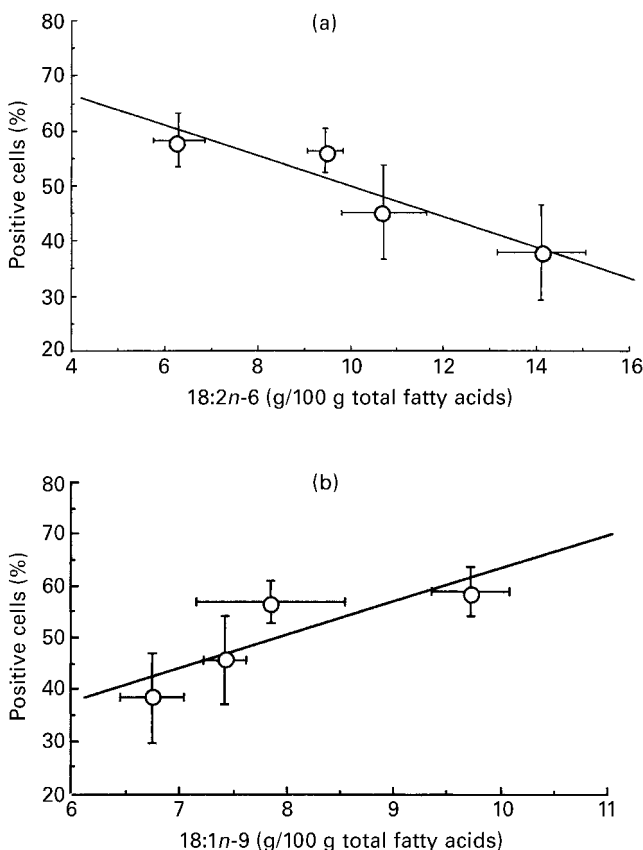


Fig. 1. Relationship between the proportion of CD25 positive spleen cells and the amount of (a) 18:2n-6 or (b) 18:1n-9 in spleen lymphocytes from rats fed on high-fat and low-fat diets containing coconut oil or soyabean oil. For details of diets, see Table 1. Values for spleen lymphocyte fatty acids are means for four rats with their standard errors represented by horizontal bars. Values for CD25 expression are means for five rats, with their standard errors represented by vertical bars. A significant negative correlation ($r = -0.932$, $P = 0.028$) was found between 18:2n-6 and CD25 expression (panel a).

Expression of interleukin-2 receptor α -chain (CD25)

CD25 expression was determined either by measuring the mean fluorescence or by calculating the percentage of CD25-positive cells. The proportion of CD25-positive cells was significantly decreased by the fat source ($P = 0.040$) but not by the fat level. Mean fluorescence was also significantly decreased by the fat source ($P = 0.021$) (Table 4).

A significant negative correlation was observed between IL-2 receptor α -chain expression and the level of 18:2n-6 in phospholipid membranes of spleen lymphocytes when CD25 was expressed as the percentage of positive cells (Fig. 1(a); $r = -0.932$, $P = 0.020$) and as the mean fluorescence (Fig. 2(a); $r = -0.973$, $P = 0.001$). A significant positive correlation was observed between IL-2 receptor α -chain expression and the level of 18:1n-9 in phospholipid membranes only when CD25 was expressed as mean fluorescence (Fig. 2(b); $r = 0.929$, $P = 0.05$).

Lymphocyte proliferation

Cell proliferation measured as the proliferation index was significantly affected by the fat source ($P = 0.001$) and by the fat level ($P = 0.005$) (Table 4). A significant negative correlation was observed between lymphocyte proliferation and the level of 18:2n-6 in phospholipid membranes of spleen lymphocytes (Fig. 3(a); $r = -0.990$, $P = 0.001$), while a significant positive correlation was observed with 18:1n-9 (Fig. 3(b); $r = 0.984$, $P = 0.001$).

Discussion

As usually observed in other tissues, the present study confirms that dietary lipids modify the fatty acid composition of rat spleen lymphocytes. Indeed, after 8 weeks, the four diets differing in fat source and fat level resulted in significant differences in the fatty acid composition of lymphocyte phospholipids. Linoleic acid was the main PUFA of all the diets, and using the HF diets led to increasing amounts of linoleic acid in lymphocyte phospholipid membranes. The different diets used had no influence on the saturated fatty acid content of lymphocyte phospholipids but there were several significant differences in levels of the monounsaturated fatty acids 16:1n-7 and 18:1n-9 between lymphocytes from rats fed on the different diets. The levels of 16:1n-7 and 18:1n-9 were greater in

rats fed on the coconut LF diet compared with the soyabean HF diet which contained the highest concentration of 18:1*n*-9. Our results showed that a high level of linoleic acid in the diet induced an increase of linoleic acid with a concomitant decrease of oleic acid in the lymphocyte phospholipid membranes. The increase of monounsaturated fatty acid is usually observed with diets low in the essential fatty acids (Holman, 1968). In this case, 16:0, 18:0, 16:1*n*-7 and 18:1*n*-9 are synthesized endogenously. However, the high level of saturated fatty acids available in the coconut-oil diets (80 g/100 g total fatty acids) did not favour increased uptake into lymphocyte phospholipids but preferentially stimulated the synthesis of monounsaturated fatty acids by increasing Δ -9 desaturase activity. The four diets used also contained increasing amounts of α -linolenic acid. In the present study 18:3*n*-3 represented less than 1 g/100 g total fatty acids in lymphocyte phospholipids. Low levels of

18:3*n*-3 are usually found in different tissues suggesting that 18:3*n*-3 is rapidly metabolized (Innis, 1991). Increasing amounts of α -linolenic acid may explain the observation that levels of 22:5*n*-3 and 22:6*n*-3 were higher in the rats fed on the soyabean HF diet.

Paradoxically, levels of arachidonic acid (20:4*n*-6) were similar in rats fed on all diets used. The level of this metabolite was preserved in spite of the low amount of its precursor, linoleic acid. We (Moussa *et al.* 1996) previously reported such preservation of arachidonic acid in some tissues of rats fed on an essential fatty acid-deficient diet for 4 weeks. In lymphocyte phospholipids, docosapentaenoic acid (22:5*n*-6) decreased in rats fed on the soyabean-oil diets compared with the coconut-oil diets. As indicated elsewhere (Innis, 1991), synthesis of 22:5*n*-6 is normally limited but is increased in the liver of essential fatty acid-deficient rats. This could explain the higher level of this

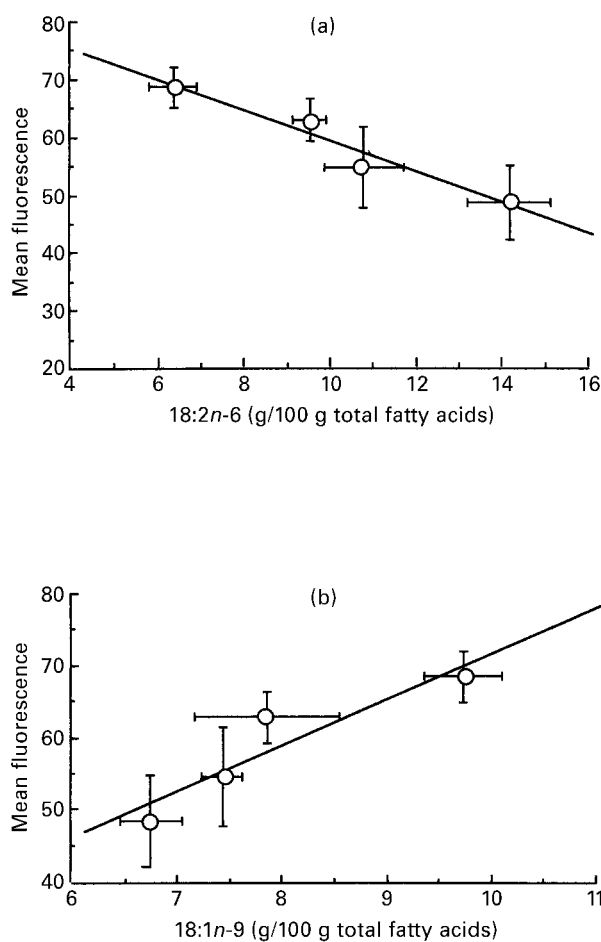


Fig. 2. Relationship between the mean fluorescence of CD25 and the amount of (a) 18:2*n*-6 or (b) 18:1*n*-9 in spleen lymphocytes from rats fed on high-fat and low-fat diets containing coconut oil or soyabean oil. For details of diets, see Table 1. Values for spleen lymphocyte fatty acids are means for four rats, with their standard errors represented by horizontal bars. Values for CD25 expression measured by fluorescence are means for five rats, with their standard errors represented by vertical bars. A significant negative correlation ($r = -0.973$, $P = 0.001$) was found between 18:2*n*-6 and CD25 expression (panel a); a significant positive correlation ($r = 0.929$, $P = 0.05$) was found between 18:1*n*-9 and CD25 expression (panel b).

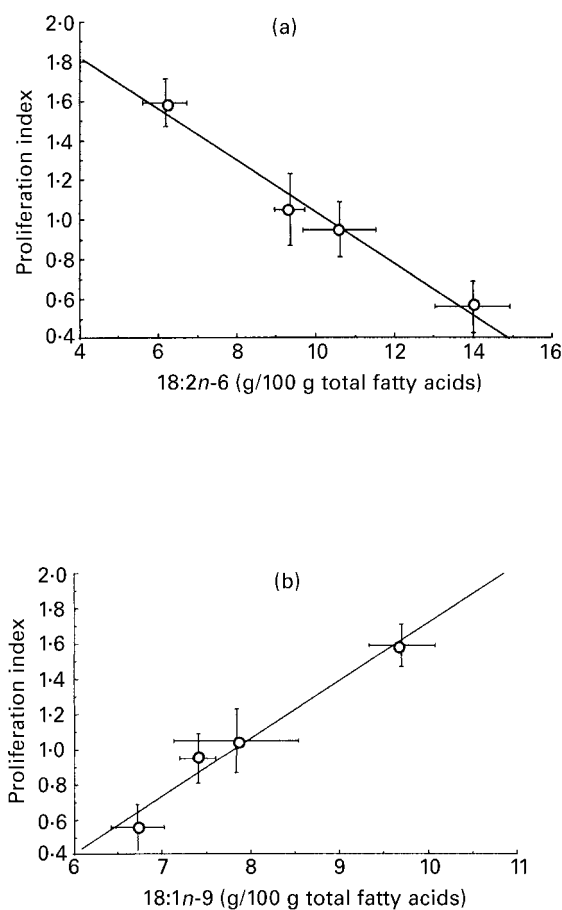


Fig. 3. Relationship between spleen lymphocyte proliferation and amount of (a) 18:2*n*-6 or (b) 18:1*n*-9 in spleen lymphocytes from rats fed on high-fat and low-fat diets containing coconut oil or soyabean oil. For details of diets, see Table 1. Values for spleen lymphocyte fatty acids are means for four rats, with their standard errors represented by horizontal bars. Values for proliferation index (see pp. 328 and 330) are means for five rats, with their standard errors represented by vertical bars. A significant negative correlation ($r = -0.990$, $P = 0.001$) was found between 18:2*n*-6 and the proliferation index (panel a); a significant positive correlation ($r = 0.984$, $P = 0.001$) was found between 18:1*n*-9 and the proliferation index (panel b).

fatty acid in lymphocyte phospholipids from animals fed on the coconut-oil diets.

The present study provides evidence that feeding animals on diets rich in unsaturated fatty acids results in inhibition of lymphocyte activation and lymphocyte proliferation. Dietary studies in which animals have been fed with PUFA-containing oils before studying the lymphocyte functions, have yielded contradictory findings. Results from such studies are difficult to compare because they differ greatly in the amount of dietary fat used. Our present results confirm that both amount and type of dietary fat play a role in the modifications observed. Furthermore, some variables were modified only by the type of fat, but none was modified only by the level of fat. This observation suggests that the effects of dietary fat are particularly linked with the fatty acid composition of the diet. In our experimental protocol, designed to investigate the effects of diets rich in linoleic acid, it was necessary to use a HF diet (soyabean HF). For comparison, we used a HF diet rich in saturated fatty acids. In most of the published studies, rats were fed on diets containing 200 g/kg of a particular lipid. The recommendations on the feeding conditions for laboratory animals suggest a diet containing 50 g lipid/kg (Potier de Courcy *et al.* 1989). Thus it was important to investigate the effects of a low level of lipid (50 g/kg diet).

Fatty acids play a structural role in biological membranes and a change in the fatty acid composition of lymphocyte membranes might influence immune functions. A significant increase in the proportion of *n*-3 PUFA in lymphocyte phospholipids was induced by the soyabean-oil diets. These modifications concerning *n*-3 PUFA could contribute to the immunosuppressive effects observed. Indeed, several studies (Yaqoob *et al.* 1994; Sanderson *et al.* 1995) have suggested that *n*-3 PUFA may be highly immunosuppressive. However, these results were obtained with diets containing fish oils, which contain high proportions of 20 : 5*n*-3 and 22 : 6*n*-3. In contrast, in the present study the increase in *n*-3 PUFA in the lymphocyte phospholipids was induced by α -linolenic acid from soyabean oil and was probably lower. Furthermore, of the PUFA, *n*-3 fatty acids are only minor components of the phospholipids of lymphocyte membranes, while linoleic acid and its derivative arachidonic acid are major components.

The proportion of arachidonic acid in lymphocyte fatty acids was not modified by the diets. From a metabolic point of view, arachidonic acid is converted to eicosanoids which are important mediators especially in immune and inflammatory responses. Our results showed that the four diets used probably did not modulate response via alterations in arachidonic acid. However, we found a significant correlation between lymphocyte function and the amount of linoleic acid present in spleen lymphocytes suggesting an immunosuppressive effect of this fatty acid. This is in agreement with other studies which have investigated the effect of diets containing *n*-6 PUFA-rich oils on immune function (Calder, 1998). Many of these show that diets very rich in linoleic acid result in suppressed lymphocyte functions. It is probably not possible to apply these findings to human diets in which the fat does not come from a single source, but in artificial nutrition, these findings would be considered of great interest. Indeed, in total parenteral

nutrition the most widely used lipid emulsions are based on soyabean oil which is rich in linoleic acid. Furthermore, a high level of lipid can be administered as part of a balanced energy supply. The immunological effect of such therapy is important since it is often administered to patients who are at risk of sepsis. Indeed, such artificial nutrition has been associated with an increased susceptibility to infection (Palmlad, 1991; Guillou, 1993; Gogos & Kalfarentzos, 1995). Our results showed that a high level of linoleic acid in the diet induced an increase in linoleic acid with a concomitant decrease in oleic acid in the phospholipid membranes. We found a significant correlation between lymphocyte function and the oleic acid present in the phospholipids of lymphocyte membranes. Our results suggest that the immunosuppressive effect of a diet rich in linoleic acid might be due to a decrease of oleic acid in the lymphocyte membrane. This highlights the beneficial role of oleic acid, a major component of olive oil which might confer health benefits, and presents a new focus of interest from a nutritional point of view. Several studies (Berger *et al.* 1993; Yaqoob *et al.* 1994; Sanderson *et al.* 1995; Jeffery *et al.* 1996) reported that feeding rats with diets containing very large amounts of olive oil significantly decreased spleen lymphocyte functions. These contradictory results could be explained by the high amount of olive oil used (200 g/kg diet). This would be equivalent to about 25 % dietary energy from oleic acid which is in excess of the amount used in the present study. Indeed, we have shown that both the amount and the type of dietary fat could play a role in the observed modifications.

Our results suggest that the immunosuppressive effect of a high-linoleic-acid diet might be due to an increase of linoleic acid or a decrease of oleic acid in the membranes of spleen lymphocytes. As to the mechanism by which an enrichment in linoleic acid or an impoverishment in oleic acid results in a decreased response of lymphocytes to mitogenic stimulation, there are several different possibilities. A change in fatty acid composition of membrane phospholipids might be expected to affect membrane fluidity and alter the function of proteins such as CD3-T-cell receptor complex involved in the early signalling pathway leading to lymphocyte activation. However, rather than a gross modification of whole membrane fluidity, 18 : 2*n*-6 enrichment or 18 : 1*n*-9 impoverishment might induce more subtle changes in specific microdomains present in the membrane. Indeed, a recent study underlined the possible functional role of these microdomains in the T-lymphocyte-signalling processes (Fra *et al.* 1994). On the other hand, the initiation of lymphocyte proliferation involves phospholipid hydrolysis to generate second messengers and changes in the membrane fatty acid composition could influence this process. Differential effects of fatty acids on diacylglycerol mass and molecular species composition have been reported with the *n*-3 PUFA (Fowler *et al.* 1993).

More studies are required to establish the effect of dietary oleic acid on immune function. Given the potentially important clinical role of oleic acid, it will be important to investigate the mechanism of this immunomodulatory action by determining how specific membrane phospholipids influenced by diet affect the components of lymphocyte signal transduction and thus can affect lymphocyte functions.

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