Dietary fatty acids influence the production of Th1- but not Th2-type cytokines

Fiona A. Wallace, Elizabeth A. Miles, Carl Evans, Theresa E. Stock, Parveen Yaqoob, and Philip C. Calder

Institute of Human Nutrition, University of Southampton, United Kingdom

Abstract: C57Bl6 mice were fed for 6 weeks on a low-fat diet or on high-fat diets containing coconut oil (rich in saturated fatty acids), safflower oil [rich in *n*-6 polyunsaturated fatty acids (PUFAs)], or fish oil (rich in *n*-3 PUFAs) as the main fat sources. The fatty acid composition of the spleen lymphocytes was influenced by that of the diet fed. Thymidine incorporation into concanavalin A-stimulated spleen lymphocytes and interleukin (IL)-2 production were highest after feeding the coconut oil diet. Interferon (IFN)-γ production was decreased by safflower oil or fish oil feeding. IL-4 production was not significantly affected by diet, although production was lowest by lymphocytes from fish oil-fed mice. The ratio of production of Th1- to Th2-type cytokines (determined as the IFN-γ/IL-4 ratio) was lower for lymphocytes from mice fed the safflower oil or fish oil diets. After 4 h of culture, IL-2 mRNA levels were higher in cells from mice fed coconut oil, and IFN-γ mRNA levels were higher in cells from mice fed coconut oil or safflower oil. After 8 h of culture, IL-2, IFN-γ, and IL-4 mRNA levels were lowest in cells from mice fed fish oil. The ratio of the relative levels of IFN-γ mRNA to IL-4 mRNA was highest in cells from mice fed coconut oil and was lowest in cells of mice fed fish oil. The influence of individual fatty acids on IL-2 production by murine spleen lymphocytes was examined *in vitro*. Although all fatty acids decreased IL-2 production in a concentration-dependent manner, saturated fatty acids were the least potent and *n*-3 PUFAs the most potent inhibitors, with *n*-6 PUFAs falling in between in terms of potency. It is concluded that saturated fatty acids have minimal effects on cytokine production. In contrast, PUFAs act to inhibit production of Th1-type cytokines with little effect on Th2-type cytokines; *n*-3 PUFAs are particularly potent. The effects of fatty acids on cytokine production appear to be exerted at the level of gene expression. *J. Leukoc. Biol.* 69: 449–457; 2001.

Key Words: *interleukin · interferon · lymphocyte · T-helper cell · fish oil · polyunsaturated fatty acid · mouse*

INTRODUCTION

Over the years there has been great interest in the effects of the different types of dietary fatty acid upon the immune system. This is partly because the long-chain *n*-6 polyunsaturated fatty acid (PUFA) arachidonic acid is the precursor of 2-series prostaglandins and 4-series leukotrienes, which have potent roles in regulating inflammation and immunity [1, 2]. Arachidonic acid is formed from a shorter chain precursor, linoleic acid, a major component of vegetable oils such as maize, sunflower, soybean, and safflower oils; intermediates in the synthesis of arachidonic acid are γ-linolenic acid and di-homo-γ-linolenic acid. The structures of the fatty acids mentioned in this article are shown in **Table 1**, while their metabolic relationships are shown in **Figure 1**. A second reason for the interest in the immunological effects of fatty acids is that epidemiological studies have found that populations such as Greenland Eskimos, whose diets contain significant quantities of the long-chain *n*-3 PUFAs eicosapentaenoic and docosahexaenoic acids, have a very low incidence of inflammatory and autoimmune disorders [3]. Fish oil (FO) is rich in these long-chain *n*-3 PUFAs, and thus it is suggested that FO is anti-inflammatory and immunomodulatory. In the absence of intake of the long-chain *n*-3 PUFAs, they can be formed from a shorter chain precursor, α-linolenic acid (Fig. 1).

Comparisons of the *in vitro* effects of different fatty acids upon lymphocyte functions were first made about 25 years ago and it was observed that linoleic and arachidonic acids were both potent inhibitors of mitogen-stimulated proliferation of rodent [4] and human lymphocytes [5–7], with arachidonic acid being the more potent. The *n*-6 PUFAs were more inhibitory than saturated fatty acids, especially palmitic acid [4, 6, 7]. Later studies showed that the *n*-3 PUFAs α-linolenic, eicosapentaenoic, and docosahexaenoic acids were also potent inhibitors of the mitogen-stimulated proliferation of rodent [8–10] and human lymphocytes [10–15]. Given the potent effects of PUFAs on T lymphocyte proliferation, it would be expected that they would alter production of T cell-derived cytokines. Indeed linoleic, α-linolenic, arachidonic, eicosapentaenoic, and docosahexaenoic acids were found to have roughly equipotent inhibitory effects on the production of interleukin (IL)-2 by concanavalin A (Con A)-stimulated rat and human lymphocytes [9, 12]; saturated fatty acids had little effect on IL-2

Present address of Parveen Yaqoob: Hugh Sinclair Unit of Human Nutrition, University of Reading, Whiteknights, Reading RG6 6AP, UK.

Correspondence: Philip C. Calder, Institute of Human Nutrition, University of Southampton, Basset Crescent East, Southampton SO16 7PX, UK. E-mail: pcc@soton.ac.uk

Received July 27, 2000; revised October 17, 2000; accepted October 20, 2000.

TABLE 1. Structures of Fatty Acids Mentioned in Text

Fatty acid name	Fatty acid structure
Capric	10:0
Lauric	12:0
Myristic	14:0
Palmitic	16:0
Stearic	18:0
Oleic	18:1*n*-9
Linoleic	18:2*n*-6
γ-Linolenic	18:3*n*-6
α-Linolenic	18:3*n*-3
Di-homo-γ-linolenic	20:3*n*-6
Arachidonic	20:4*n*-6
Eicosapentaenoic	20:5*n*-3
Docosapentaenoic	22:5*n*-3
Docosahexaenoic	22:6*n*-3

production. More recently, Purasiri et al. [14] have confirmed that eicosapentaenoic and docosahexaenoic acids inhibit IL-2 production by cultured human lymphocytes.

The effects of fatty acids on lymphocyte proliferation that have been observed *in vitro* have been confirmed in feeding experiments, although these are often difficult to compare because of the levels of fat used and the exact comparison being made. Several studies show that feeding rats or mice diets rich in saturated fat has little effect on lymphocyte proliferation compared with feeding a low-fat diet [16–18], although some studies do show a suppressive effect of high saturated fat feeding, but this is less pronounced than the effect of feeding high levels of PUFAs [19–23]. Feeding laboratory rodents diets rich in linoleic acid, α-linolenic acid, or FO leads to decreased mitogen-stimulated lymphocyte proliferation examined *ex vivo* [10, 16, 19, 20, 22–27]. It appears that FO is more potent than α-linolenic acid-rich oils which are, in turn, more potent than linoleic acid-rich oils. Increasing the amount of α-linolenic acid or FO in the human diet has been shown to lead to a decreased proliferative response of blood lymphocytes [28–30]. Compared with the large number of studies of the influence of dietary fat on lymphocyte proliferation there are relatively few studies of the influence on T cell-derived cytokines. Turek et al. [31] reported decreased IL-2 production by alveolar lymphocytes after feeding pigs diets rich in α-linolenic acid or FO. More recently, eicosapentaenoic and docosahexaenoic acids were shown to be equipotent in their ability to decrease IL-2 production when included in the mouse diet at very high levels [32]. Increasing the amount of FO in the human diet decreased *ex vivo* IL-2 production by blood lymphocytes [29, 30, 33, 34]. There are few studies of dietary fatty acids and lymphocyte-derived cytokines other than IL-2. Gallai et al. [34] reported decreased interferon (IFN)-γ production by Con A-stimulated blood lymphocytes after FO supplementation of the human diet. In contrast to these studies, there were no differences in the *ex vivo* production of IL-2, IFN-γ, IL-4, or IL-10 among spleen lymphocytes from mice fed diets rich in saturated fatty acids, *n*-6 PUFAs, and *n*-3 PUFAs [24]. There are few other studies investigating dietary fatty acids and T cell-derived cytokine production.

Given the key role of T helper (Th)1-type and Th2-type cytokines in mounting appropriate immune responses to pathogens and also in human disease [35, 36], it seems important to understand more fully the influence of dietary fatty acids in modulating the production of both Th1-type and Th2-type cytokines. Therefore, in the current study we investigated the production of Th1- and Th2-type cytokines and their mRNAs by murine spleen lymphocytes taken from mice fed diets rich in saturated fatty acids, *n*-6 PUFA and *n*-3 PUFA. In addition, we made a detailed comparison of the *in vitro* effects of fatty acids on IL-2 production by these cells.

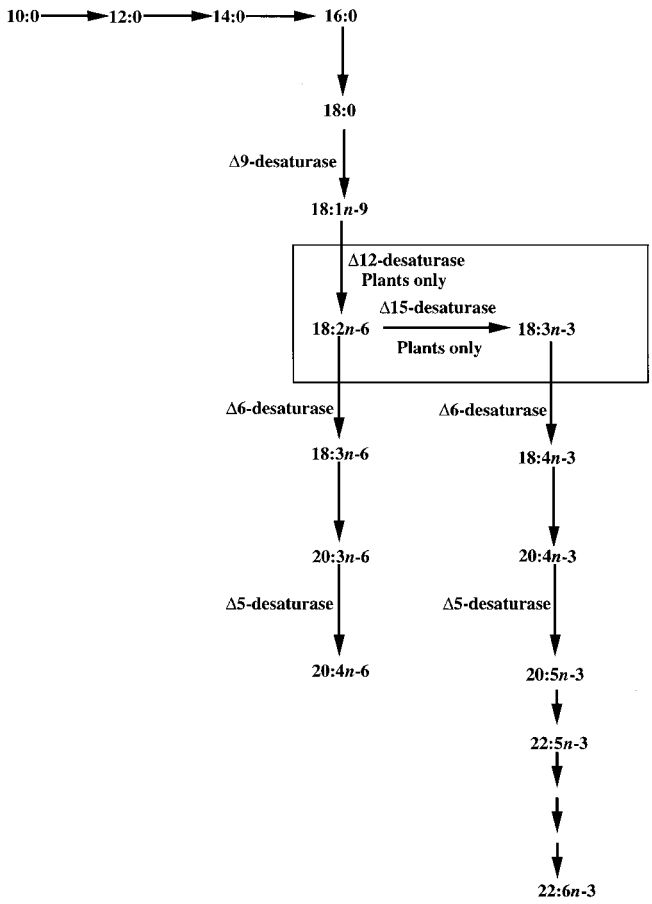

Fig. 1. Metabolic relationships among various fatty acids.

MATERIALS AND METHODS

Animals and diets

Male C57Bl6 mice (6 weeks old and weighing ~20 g) were purchased from Charles River (Margate, Kent, UK). They were housed in plastic cages in controlled environmental conditions (21.5 ± 0.5°C, 45 ± 2% humidity; 14 h light/10 h dark cycle). Mice used for preliminary studies and as a source of cells for *in vitro* experiments were fed on standard laboratory chow (Rat and Mouse No. 1 pellets; Special Diet Services, Witham, Essex, UK). For the dietary studies mice were randomly allocated to receive one of four diets on which they were maintained for 6 weeks until they were killed. The diets used were a low-fat (LF) diet and three high-fat (HF) diets (all prepared by ICN Biomedicals, High Wycombe, Bucks., UK); the LF diet most closely resembled standard mouse chow and contained 25 g maize oil/kg. The HF diets contained 200 g/kg of the lipid under study [coconut oil (CO), safflower oil (SO), or FO], plus 10 g maize oil/kg to prevent essential fatty acid deficiency. All diets contained 200 g protein, 200 g starch, 296 g sucrose, and 1.2 g vitamin E (250 IU/g) per kg and all diets were in pelleted form. In the HF diets the additional

TABLE 2. Fatty Acid Composition of the Diets Used

Diet	Fatty acid (g/100 g total fatty acids)										
	10:0	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-3	20:5*n*-3	22:6*n*-3
Chow	n.d.	n.d.	1.2	20.5	0.3	4.5	16.1	50.1	2.8	n.d.	0.3
LF	n.d.	3.4	3.5	13.7	n.d.	3.3	21.9	54.3	n.d.	n.d.	n.d.
CO	6.8	56.5	17.6	7.8	n.d.	5.1	2.0	2.3	n.d.	n.d.	n.d.
SO	1.6	4.3	1.1	8.7	n.d.	3.9	19.4	61.0	n.d.	n.d.	n.d.
FO	n.d.	n.d.	10.3	22.0	14.5	4.2	11.8	9.0	3.5	10.6	10.1

n.d., not detected.

fat was accompanied by a decrease in the amount of fiber compared with the amount in the LF diet (50 g/kg in the HF diets vs. 225 g/kg in the LF diet). The fatty acid composition of the diets is shown in **Table 2**. The CO diet was rich in the medium-chain saturated fatty acids capric, lauric, and myristic acid. The chow, LF, and SO diets were rich in the *n*-6 PUFA linoleic acid. The FO diet contained the long-chain *n*-3 PUFA eicosapentaenoic and docosahexaenoic acid. Apart from the FO diet, which was stored frozen, all diets were stored at 20°C. Diets were provided fresh to the animals every 2 days. Mice were killed between 0800 and 0930 h by a rising concentration of CO_2. All procedures involving experimental animals were approved under the Animals (Scientific Procedures) Act 1986 by the Home Office.

Chemicals

General laboratory chemicals, glutamine, fetal calf serum (FCS), streptomycin, penicillin, Con A, Histopaque, RPMI 1640 culture medium, all fatty acids, diethylpyrocarbamate (DEPC), agarose, and ethidium bromide were purchased from Sigma Chemical (Poole, Dorset, UK). [^{3}H]thymidine (5 Ci/mmol) was purchased from Amersham International, Amersham, Bucks., UK. Cytokine enzyme-linked immunosorbent assay (ELISA) kits were purchased from BioSource International, Camarillo, CA. TRIzol was purchased from Life Technologies, Paisley, UK. Triton X-100, avian myeloblastosis virus reverse transcriptase, Taq polymerase and poly(dT)$_{15}$ were purchased from Promega, Southampton, Hants., UK. Deoxyribonucleosidetriphosphates (dNTPs) were purchased from Pharmacia, Milton Keynes, Beds., UK.

Spleen lymphocyte preparation

Spleens were removed immediately after death and were placed in RPMI culture medium. The cells were released by teasing apart the spleens through a wire mesh strainer with the end of a 10-mL plastic syringe plunger. Cell preparations were filtered through lens tissue to remove debris and the cells collected by centrifugation (500 *g*, 5 min). The pelleted cells were resuspended and mononuclear cells isolated by centrifugation on Histopaque (1000 *g*, 20 min). Mononuclear cells were collected from the interface and were washed in RPMI culture medium. Finally, they were resuspended at a concentration of 2×10^6 cells/mL in RPMI culture medium containing 2 mM glutamine, 5% (vol/vol) FCS, and antibiotics (50 μg/mL penicillin plus 50 μg/mL streptomycin).

Lymphocyte proliferation

Spleen lymphocytes (2×10^5) were cultured at 37°C in an atmosphere of 19:1 air/CO_2 in RPMI culture medium containing 2 mM glutamine, 5% (vol/vol) FCS, antibiotics and, for stimulated cells, 2.5 μg/mL Con A [preliminary experiments indicated that this was the concentration of Con A that induced maximal proliferation of spleen lymphocytes from C57Bl6 mice of this age (data not shown)]; the final volume of the culture was 200 μL. After 48 h 20 μL [^{3}H]thymidine (0.1 μCi) was added to each well and the cells were cultured for a further 18 h. Then the contents of the wells were transferred to glass fiber filters and washed and dried using a Skatron Cell Harvester (Skatron, Lier, Norway). The filters were transferred to counting vials and 100 μL scintillant (Wallac Optiphase Hisafe purchased from Fisher Scientific, Loughborough, Leics., UK) added to each; radioactive incorporation was measured using a Beckman LS6500 liquid scintillation counter. Lymphocyte proliferation data are expressed as [^{3}H]thymidine incorporation in cpm/well.

Lymphocyte culture for cytokine analysis

Spleen lymphocytes (2×10^6) were cultured at 37°C in an atmosphere of 19:1 air/CO_2 in RPMI culture medium containing 2 mM glutamine, 5% (vol/vol) FCS, antibiotics, and 2.5 μg/mL Con A; the final volume of the culture was 2 mL. Preliminary experiments indicated that a Con A concentration of 2.5 μg/mL induced maximal production of each of the cytokines studied (data not shown). Cells from dietary experiments were cultured for 4 and 8 h (for cytokine mRNA analysis) and for 24 h (for measurements of cytokine concentrations in the culture medium). For *in vitro* studies of the effects of individual fatty acids, spleen lymphocytes were cultured in the same conditions except that the medium also contained fatty acids at final concentrations of 5, 10, 25, or 50 μM. For these experiments cells were cultured for 48 h. At the end of the culture period the culture plates were centrifuged to separate the cells from the medium. Aliquots of the medium were frozen at −70°C for cytokine analysis. The concentrations of the cytokines in the culture medium were measured by commercially available ELISA kits; all measurements were made according to the instructions given by the manufacturers of the ELISA kits.

Extraction of RNA, reverse transcription, and DNA amplification and visualization

Total RNA was extracted using TRIzol in accordance with the manufacturer's instructions. mRNA was then selectively reverse transcribed from 4.5 μg of total RNA using an oligo(dT) primer. Reverse transcription was achieved with 7.5 U of avian myeloblastosis virus reverse transcriptase in the presence of 1 mM dNTPs, 5 mM magnesium chloride, and 0.5 μg poly(dT)$_{15}$; the reaction was buffered by 10 mM Tris, 50 mM potassium chloride, 0.1% (v/v) Triton X-100, pH 8.8, and the total volume was 20 μL. Reverse transcription was carried out for 1 h at 42°C followed by heating at 94°C for 3 min to inactivate the transcriptase. The resulting cDNA was diluted with DEPC-treated water to a final volume of 35 μL and used as a polymerase chain reaction (PCR) substrate.

PCR was performed for a housekeeping gene (cyclophilin), IL-2, IFN-γ, and IL-4. Amplification of 2.5 μL of cDNA was achieved using 1 U of Taq polymerase in the presence of 15 pmol of primer (primer sequences are shown in **Table 3**), magnesium-free buffer [19 mM Tris, 50 mM potassium chloride, 0.1% (v/v) Triton X-100, pH 9], 2 mM magnesium chloride (1.5 mM for PCR of cyclophilin) and 0.2 mM dNTPs. The reaction mix was incubated at 93°C for 1 min. The reaction was cycled at 94°C for 30 s, the annealing temperature (see Table 3) for 30 s, and 72°C for 1 min in a Hybaid Touchdown Thermocycler (Hybaid, Teddington, Middlesex, UK). The optimized number of cycles used (reflecting the exponential phase of the reaction) is shown in Table 3.

PCR products were electrophoresed in 2% agarose gels stained with ethidium bromide. The resultant bands were visualized with an ultraviolet transilluminator and the image stored with a GDS 5000 gel documentation system (UVP, Cambridge, Cambs., UK). The images were then analyzed by densitometry using Phoretix 2D 4.00 software (Phoretix International, Newcastle-Upon-Tyne, Tyne & Wear, UK). All results are expressed as the ratio of cytokine mRNA to cyclophilin mRNA where the cDNA was amplified for cyclophilin concurrently with amplification for cytokines.

Fatty acid composition analysis

Total lipid was extracted from lymphocytes with chloroform/methanol (2:1 v/v) as previously described [37]. Fatty acids were prepared by saponification for

TABLE 3. Details of PCR Conditions

cDNA to be amplified	Primer sequences	Annealing temperature (°C)	Number of cycles
Cyclophilin	Sense: 5′-TTGGGTCGCGTCTCGTTCGA-3′ Anti-sense: 5′-GCCAGGACCTGTATGCTTCA-3′	60	30
IL-2	Sense: 5′-ACTTCAAGCTCCTCCACTTCAAGC-3′ Anti-sense: 5′-GCTTTGAGAAAGGGCTATCCA-3′	60	36
IFN-γ	Sense: 5′-AACGCTTACACACTGCATCTTGG-3′ Anti-sense: 5′-GACTTCAAAGAGTCTAGAG-3′	60	30
IL-4	Sense: 5′-GAATGTACCAGCAGCCATATC-3′ Anti-sense: 5′-CTCAGTACTACGAGTAATCCA-3′	58	38

3 h at 80°C in methanolic 0.5 M sodium hydroxide; after cooling the samples were neutralized by addition of 5 M sulfuric acid. Then, fatty acids were extracted with chloroform/methanol (2:1 v/v) and fatty acid methyl esters prepared by incubation with diazomethane in ether. Fatty acid methyl esters were isolated by solvent extraction, dried, and separated by gas chromatography in a Hewlett-Packard 6890 gas chromatograph (Hewlett Packard, Avondale, PA) fitted with a 30 m × 0.32 mm BPX70 capillary column, film thickness 0.25 μm. Helium at 2.0 mL/min was used as the carrier gas and the split/splitless injector was used with a split:splitless ratio of 10:1. Injector and detector temperatures were 170 and 250°C, respectively. The column oven temperature was maintained at 170°C for 12 min after sample injection and was programmed to then increase from 170 to 200°C at 5°C/min before being maintained at 200°C for 15 min. The separation was recorded with Hewlett Packard gas chromatography Chem Station software (Hewlett Packard, Avondale, PA). Fatty acid methyl esters were identified by comparison with standards run previously.

Statistical analysis

All data are presented as means ± SE. Statistically significant differences between dietary groups or between cultures containing different fatty acids were determined by one-way analysis of variance and a *post-hoc* least-significant difference test. All analyses were performed using SPSS version 6.0 (SPSS, Chicago, IL) and a value for $P < 0.05$ was taken to indicate a statistically significant difference.

RESULTS

Dietary fatty acids and spleen fatty acid composition

The fatty acid composition of spleen lymphocytes was markedly influenced by that of the diet fed (**Table 4**). Lymphocytes from mice fed the CO diet were significantly enriched in myristic acid, compared with those from mice fed each of the other diets (Table 4). Lymphocytes from mice fed the SO diet were significantly enriched in some of the derivatives of linoleic acid (Table 4). Lymphocytes from mice fed the FO diet contained significantly higher proportions of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids than lymphocytes from mice fed each of the other diets (Table 4). The incorporation of these fatty acids was associated with significant decreases in the proportions of *n*-6 PUFAs (Table 4). The total proportion of *n*-6 PUFAs was 20–25% in lymphocytes from mice fed the LF, CO, or SO diets; in contrast *n*-6 PUFAs contributed only 12% of fatty acids in the lymphocytes from mice fed the FO diet. The *n*-6/*n*-3 PUFA ratio in spleen lymphocyte lipids was between 75 and 100 after feeding the LF or CO diets, approximately 10 after feeding the SO diet, but approximately 0.7 after feeding the FO diet.

Dietary fatty acids and spleen lymphocyte proliferation

Thymidine incorporation into Con A-stimulated spleen lymphocytes was greater after feeding the CO diet than after feeding each of the other diets (**Fig. 2**).

Dietary fatty acids and cytokine production by spleen lymphocytes

IL-2 production was greater after feeding the CO diet than after feeding each of the other three diets (**Table 5**). IFN-γ production was decreased by SO or FO feeding compared with LF or CO feeding (Table 5). IL-4 production was not significantly affected by diet, although production was lowest by lymphocytes from FO-fed mice (Table 5). The ratio of production of Th1- to Th2-type cytokines was determined as the IFN-γ/IL-4 ratio. This ratio was significantly lower for lymphocytes from mice fed the SO or FO diets compared with those from mice fed the LF or CO diets (Table 5).

Dietary fatty acids and cytokine mRNA levels in spleen lymphocytes

IL-2 mRNA levels increased between 4 and 8 h of culture (**Fig. 3A**). After 4 h of culture, IL-2 mRNA levels tended to be higher in cells from mice fed CO compared with those from FO-fed mice (Fig. 3A). After 8 h of culture, IL-2 mRNA levels were lowest in cells from mice fed FO; indeed in these cells IL-2 mRNA levels were not increased at 8 h compared with 4 h (Fig. 3A).

IFN-γ mRNA levels increased between 4 and 8 h of culture (Fig. 3B). After 4 h of culture, IFN-γ mRNA levels tended to be higher in cells from mice fed CO or SO (Fig. 3B). After 8 h of culture IFN-γ mRNA levels were lowest in cells from mice fed FO; in these cells IFN-γ mRNA levels were not increased at 8 h compared with 4 h (Fig. 3B).

IL-4 mRNA levels were similar after 4 and 8 h of culture (Fig. 3C). After 4 h of culture IL-4 mRNA levels tended to be higher in cells from mice fed SO (Fig. 3C). After 8 h of culture IL-4 mRNA levels were lowest in cells from mice fed FO (Fig. 3C).

The ratio of the relative levels of IFN-γ mRNA to IL-4 mRNA was higher at 4 h of culture in cells from mice fed CO (approximately 0.5) than in those from mice fed the other three diets (approximately 0.2 to 0.3). At 8 h of culture this ratio was lower in cells of mice fed FO (approximately 0.3) compared with those fed the other three diets (approximately 0.6–0.8).

TABLE 4. Fatty Acid Composition of Spleen Lymphocytes from Mice Fed Different Diets

Diet	Fatty acid (g/100 g total fatty acids)												
	12:0	14:0	16:0	16:1*n*-7	18:0	18:1*n*-9	18:2*n*-6	18:3*n*-6	20:2*n*-6	20:3*n*-6	20:4*n*-6	20:5*n*-3	22:5*n*-3	22:6*n*-3
LF	3.1 ± 0.2	2.4 ± 0.7^{b}	27.3 ± 1.1^{a}	2.4 ± 0.4^{b}	14.0 ± 1.8^{b}	9.6 ± 1.1^{ab}	5.6 ± 0.1^{a}	<0.1^{b}	2.4 ± 0.6^{b}	1.5 ± 0.1^{bc}	13.7 ± 0.9^{a}	<0.1^{b}	<0.1^{b}	<0.1^{c}
CO	3.2 ± 0.7	6.9 ± 1.0^{a}	29.3 ± 0.2^{a}	3.3 ± 0.1^{ab}	14.9 ± 0.5^{b}	10.5 ± 0.5^{a}	5.4 ± 0.5^{a}	<0.1^{b}	3.9 ± 0.5^{ab}	2.1 ± 0.1^{b}	12.7 ± 0.7^{a}	<0.1^{b}	<0.1^{b}	<0.1^{c}
SO	3.0 ± 0.2	2.9 ± 0.4^{b}	23.5 ± 1.8^{b}	2.9 ± 0.3^{b}	20.4 ± 4.0^{a}	6.4 ± 0.5^{c}	4.3 ± 0.7^{a}	2.2 ± 0.1^{a}	4.3 ± 0.7^{a}	3.3 ± 0.1^{a}	7.2 ± 2.4^{b}	<0.1^{b}	<0.1^{b}	2.2 ± 0.1^{b}
FO	3.0 ± 0.2	2.8 ± 0.4^{b}	30.9 ± 2.7^{a}	4.5 ± 0.4^{a}	5.0 ± 1.4^{c}	8.6 ± 1.9^{b}	1.9 ± 0.6^{b}	<0.1^{b}	1.9 ± 0.6^{c}	0.9 ± 0.1^{c}	6.9 ± 1.0^{b}	4.6 ± 0.9^{a}	3.7 ± 0.4^{a}	8.1 ± 1.5^{a}

Data are means ± SE for six mice fed on each diet. Values in a column not sharing a common superscript letter are significantly different ($P < 0.05$; one-way analysis of variance).

Fig. 2. Proliferation of Con A-stimulated spleen lymphocytes from mice fed different diets. Data are means ± SE for six mice fed each diet. *Significantly different from each of the other diets ($P<0.05$; one-way ANOVA).

In vitro effects of fatty acids on cytokine production by spleen lymphocytes

All fatty acids decreased IL-2 production in a concentration-dependent manner, although the effect of palmitic acid did not reach statistical significance (**Table 6**). Saturated fatty acids were relatively poor inhibitors of IL-2 production (Table 6). The shorter-chain *n*-6 PUFAs, linoleic and γ-linolenic acids, were also relatively poor inhibitors causing 55% inhibition at a concentration of 50 μM (Table 6), with an IC_{50} value of approximately 47 μM. The long-chain *n*-6 PUFAs, di-homo-γ-linolenic and arachidonic acids, were more potent, causing 75% inhibition at a concentration of 50 μM (Table 6) with an IC_{50} of approximately 22 μM. Oleic and docosahexaenoic acids had IC_{50} values of approximately 32 to 35 μM, although docosahexaenoic acid caused greater inhibition (95%) than oleic acid (70%) at a concentration of 50 μM (Table 6). α-Linolenic acid was the most potent inhibitor, causing 96% inhibition at a concentration of 50 μM (Table 6) with an IC_{50} of approximately 16 μM. Eicosapentaenoic acid exhibited a unique pattern of effects: this fatty acid tended to enhance IL-2 production at the lowest concentrations tested (5 and 10 μM) and only became inhibitory at the highest concentration tested (50 μM), at which it caused almost 80% inhibition of IL-2 production (Table 6).

TABLE 5. Cytokine Production by Con A-Stimulated Spleen Lymphocytes from Mice Fed Different Diets

Diet	Concentration (pg/mL)			
	IL-2	IFN-γ	IL-4	IFN-γ/IL-4
LF	137 ± 25^{b}	7.1 ± 2.7^{a}	56 ± 8	0.13 ± 0.03^{a}
CO	231 ± 44^{a}	9.1 ± 1.0^{a}	57 ± 10	0.16 ± 0.05^{a}
SO	106 ± 8^{b}	3.1 ± 1.3^{b}	67 ± 12	0.05 ± 0.01^{b}
FO	137 ± 19^{b}	1.3 ± 1.0^{b}	48 ± 13	0.03 ± 0.02^{b}

Data are means ± SE for six mice fed on each diet. Values in a column not sharing a common superscript letter are significantly different ($P<0.05$; one-way ANOVA).

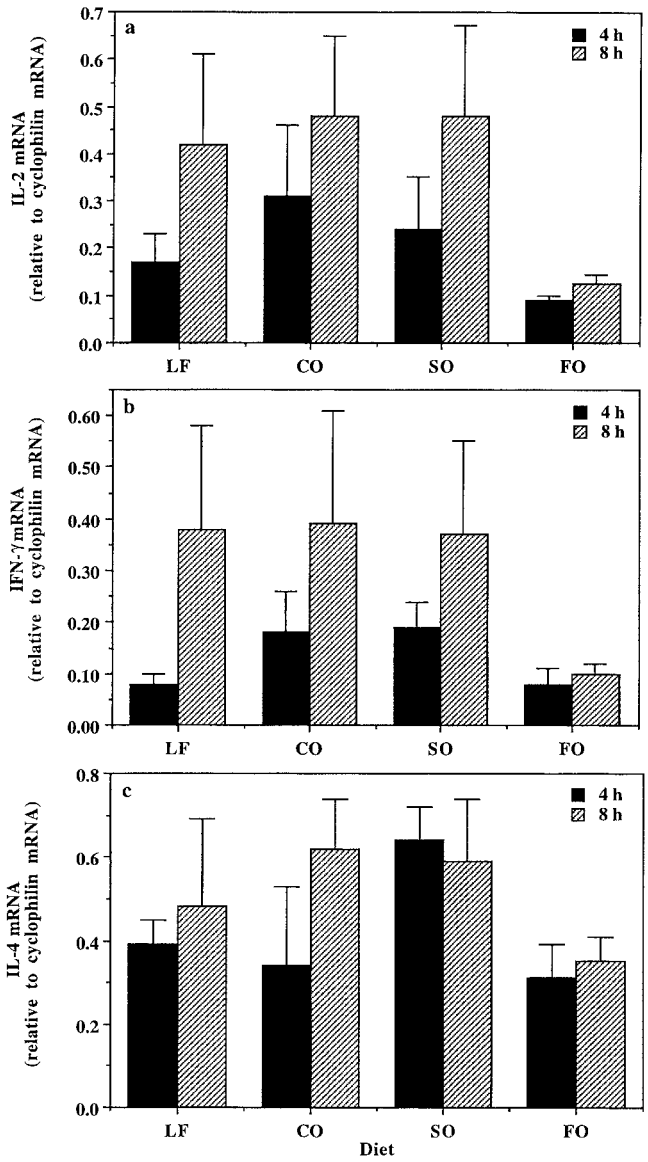

Fig. 3. Cytokine mRNA levels in Con A-stimulated spleen lymphocytes from mice fed different diets. The levels of mRNA for IL-2 (A), IFN-γ (B), and IL-4 (C) after 4 and 8 h of culture are expressed relative to the levels of mRNA for the housekeeping gene cyclophilin. Data are means ± SE for three or four mice fed each diet.

DISCUSSION

The fatty acid composition of spleen lymphocytes from mice fed the CO diet, characterized mainly by an increase in the proportion of myristic acid, is consistent with that reported previously for spleen lymphocytes from rats fed this diet [38]. Likewise the fatty acid composition of spleen lymphocytes from mice fed the FO diet, characterized by increased proportions of eicosapentaenoic, docosapentaenoic, and docosahexaenoic acids and decreased proportions of linoleic and arachidonic acids, is consistent with that reported for spleen lymphocytes from rats fed this diet [38]. The enrichment of blood lymphocytes with the long-chain *n*-3 PUFAs, partly at the expense of arachidonic acid, is also observed in humans who consume increased amounts of FO [39, 40]. In contrast to these consistencies with rat and human studies, the fatty acid composition of spleen lymphocytes from mice fed the SO diet, in which the proportion of arachidonic acid was decreased, was different from that observed previously in the spleen lymphocytes of rats fed this diet [38]. Rat lymphocytes showed increased proportions of both linoleic and arachidonic acids after feeding the SO diet [38], whereas murine lymphocytes did not show enrichment in linoleic acid and showed a decreased proportion of arachidonic acid. These observations suggest either that the metabolism of linoleic acid is different between rats and mice or that the regulation of the incorporation of *n*-6 PUFAs into lymphocytes is different between rats and mice.

The pattern of effects of the diets on spleen lymphocyte proliferation stimulated by Con A is consistent with that previously shown for spleen lymphocytes from MF1 strain mice fed these diets [24]. In that study the proliferation of lymphocytes from mice fed on CO was greater than that of those from mice fed on the LF and SO diets, which were, in turn, greater than that of lymphocytes from mice fed on FO [24]. Spleen lymphocytes from rats fed the SO or FO diets proliferated less well than those from rats fed the LF or CO diets (when the cells were cultured in FCS, as in the current study), although CO did not enhance rat lymphocyte proliferation compared with the LF diet [22]. Taking these studies together, it appears that, in comparison with saturated fatty acids and as components of a HF diet, long-chain *n*-3 PUFAs at the levels present in the FO

TABLE 6. IL-2 Production by Spleen Lymphocytes Incubated with Various Fatty Acids

	Fatty acid concentration			
Fatty acid	5 μM	10 μM	25 μM	50 μM
Palmitic	678 ± 130	555 ± 121	546 ± 116	518 ± 126^{a}
Stearic	746 ± 93	578 ± 95	519 ± 107	386 ± 151*^{a}
Oleic	679 ± 119	588 ± 100	452 ± 78	235 ± 92*^{b}
Linoleic	732 ± 118	695 ± 105	652 ± 96^{a}	353 ± 79*^{b}
γ-Linolenic	636 ± 165	542 ± 121^{b}	528 ± 164	351 ± 96*^{b}
α-Linolenic	621 ± 149^{b}	471 ± 134^{b}	277 ± 142*^{b}	34 ± 26*^{d}
Di-homo-γ-linolenic	616 ± 158^{b}	501 ± 133^{b}	373 ± 82*	184 ± 31*^{c}
Arachidonic	599 ± 149^{b}	466 ± 144^{b}	369 ± 131*	186 ± 64*^{c}
Eicospentaenoic	1171 ± 414^{a}	1082 ± 436^{a}	688 ± 103^{a}	170 ± 69*^{c}
Docosahexaenoic	658 ± 119	687 ± 129	597 ± 123	42 ± 2*^{d}

IL-2 production is in ng/mL. Data are means ± SE for separate cell preparations from three mice fed on standard chow. * Significantly different from no fatty acid added control (mean ± SE = 790 ± 90 ng/mL; $P<0.05$; one-way ANOVA). Values down a column not sharing a common superscript letter are significantly different ($P<0.05$; one-way ANOVA).

diet (i.e., about 20% of dietary fatty acids) are approximately as effective at inhibiting lymphocyte proliferation as is linoleic acid at the level at which it is present in SO (i.e., about 60% of dietary fatty acids). The differences in the proportions of the *n*-3 PUFAs and linoleic acid in these diets suggest that long-chain *n*-3 PUFAs are about three times more potent at inhibiting lymphocyte proliferation than is linoleic acid. This potency of long-chain *n*-3 PUFAs is consistent with the inhibition of lymphocyte proliferation observed when humans supplement their diet with FO [29, 30]. The similar effects of SO and FO upon lymphocyte proliferation reported in the current study and previously [22, 24], appear to contrast with the observations of Jolly et al. [32]. Those authors reported that feeding C57Bl6 mice on diets rich in either eicosapentaenoic acid or docosahexaenoic acid significantly decreased Con A-stimulated spleen lymphocyte proliferation (by ~80%) compared with feeding SO. However, in the study by Jolly et al. [32] the long-chain *n*-3 PUFAs contributed 32% of dietary fatty acids, which is a 50% increase compared with their proportion in the FO diet used in the current study.

The pattern of effects of the diets on IL-2 production in response to Con A was similar to the effects on lymphocyte proliferation: the CO diet increased IL-2 production compared with the other diets. There was a strong positive correlation between IL-2 production and thymidine incorporation ($r = 0.85$), as might be expected given the regulatory role of IL-2 in lymphocyte proliferation [41], and suggesting that the effect of dietary fatty acids on lymphocyte proliferation is mediated via changes in IL-2 production. Several studies in humans have reported that increased consumption of FO leads to decreased *ex vivo* IL-2 production by blood lymphocytes [29, 30, 33, 34]. However, to our knowledge there are only two animal studies demonstrating this, one in pigs [31] and one in mice [32]. The similar effects of SO and FO upon IL-2 production observed in the current study appear to contrast with those of Jolly et al. [32] who reported that feeding C57Bl6 mice on diets rich in either eicosapentaenoic acid or docosahexaenoic acid significantly decreased IL-2 production by Con A-stimulated spleen lymphocytes (by ~50%) compared with feeding SO. However, as described above, this difference might relate to the difference in the contribution of long-chain *n*-3 PUFAs to the diet between the studies. One other animal study reported IL-2 production by murine spleen lymphocytes after feeding the diets used in the current study [24]. That study found no significant effects of diet. The reason for the difference between the earlier study [24] and the current study is not clear, although it might relate to the strain of mouse used: the earlier study used MF1 mice.

IL-2 mRNA was more rapidly induced in Con A-stimulated spleen lymphocytes from mice fed CO compared with those fed the LF or FO diets. This might account for the higher IL-2 production by cells from CO-fed mice compared with those from mice fed the LF or FO diets. Thus, the enhancing effect of CO on IL-2 production might relate to more rapid induction of gene transcription. IL-2 mRNA levels in lymphocytes from SO-fed mice were similar to those in cells from mice fed the LF or CO diets, and so the reason for decreased IL-2 production by these cells, in comparison to those from CO-fed mice, is not clear. SO feeding may affect some later component of IL-2 processing. Jolly et al. [42] studied IL-2 mRNA levels in Con-A-stimulated spleen lymphocytes from C57Bl6 mice fed on diets rich in SO or long-chain *n*-3 PUFAs. They stated that dietary *n*-3 PUFAs do not affect IL-2 gene expression [42]. However, the data shown indicate that IL-2 mRNA levels were significantly lower at 3 and 9 h of culture in cells from mice fed long-chain *n*-3 PUFAs [42].

Reports of dietary fats and production of cytokines other than IL-2 by lymphocytes are rare. Gallai et al. [34] reported decreased IFN-γ production by blood lymphocytes from humans who consumed FO. This ability of FO to decrease IFN-γ production by lymphocytes, at least in comparison with the LF and CO diets, is demonstrated in the current study. IFN-γ mRNA was slightly more rapidly induced in Con A-stimulated spleen lymphocytes from mice fed CO compared with those fed the LF diet. This might account for the slightly higher IFN-γ production by cells from CO-fed mice compared with those from mice fed the LF diet. IFN-γ mRNA was very poorly expressed in cells from FO-fed mice. This most likely accounts for the very low production of IFN-γ by these cells. Thus, the inhibiting effect of FO on IFN-γ production might relate to markedly decreased induction of gene transcription. IFN-γ mRNA levels in lymphocytes from SO-fed mice were similar to those in cells from mice fed the LF or CO diets, and so the reason for decreased IFN-γ production by these cells, in comparison to those from LF or CO-fed mice, is not clear. SO feeding may affect some later component of IFN-γ processing.

IL-4 production was not affected by the diets used here, which is in agreement with an earlier study in MF1 mice [24], although they tended to be highest in mice fed SO and lowest in mice fed FO. IL-4 mRNA was more rapidly induced in the cells of SO-fed mice and was relatively poorly induced in the cells of FO-fed mice. Thus, the moderate effects of dietary fatty acids on IL-4 production appear to be related to effects at the level of IL-4 gene expression.

The effects of dietary fats on the production of cytokines and their mRNAs suggest that they are able to alter the Th1-/Th2-type cytokine balance. FO appears to be particularly potent at skewing this balance away from Th1 toward Th2. Compared with CO feeding, FO altered the balance in production of cytokines away from Th1 and toward Th2 by a factor of about 5; at the level of the cytokine mRNAs FO altered this balance by a factor of about 2. These data can be compared with those from a study in which the levels of mRNA for IFN-γ and IL-10 were measured in the Peyer's patches of diabetes-prone rats fed diets rich in either saturated fat or FO [43]. The FO diet decreased the level of IFN-γ mRNA by about 65% and increased the level of IL-10 mRNA by about 5.5-fold, so causing a shift in the Th1/Th2 balance (defined as IFN-γ mRNA/IL-10 mRNA) away from Th1 by a factor of about 15 [43].

Although there have been extensive *in vitro* studies of the influence of individual fatty acids on lymphocyte proliferation [4–15], there have been relatively few such studies on cytokines. In our previous studies we reported the effects of fatty acids at a concentration of 100 μM on IL-2 production by rat [9] or human [12] lymphocytes. This is a higher concentration of unesterified fatty acid than is likely to occur in the bloodstream or intracellularly. In the current study fatty acid concentrations of 5–50 μM were used. As a group, the *n*-3 PUFAs

were the most potent inhibitors of IL-2 production *in vitro*, whereas, as a group, the *n*-6 PUFAs were more potent than the saturated fatty acids. Thus, the relative potencies of the fatty acid families as inhibitors of IL-2 production *in vitro* mirror the relative potencies observed when diets rich in those particular fatty acids are fed to mice.

Thus, *n*-3 PUFAs provided to spleen lymphocytes either *in vitro* or in the diet result in decreased cytokine production by lymphocytes, with the strongest effects being observed upon Th1 cytokines. These effects occur at both the mRNA and the secreted protein levels, and they contrast with the effects of saturated fatty acids. The decreased induction of mRNA for all three cytokines studied suggests that FO feeding results in a defect early in the signaling pathway which links cellular activation to cytokine gene induction. Con A binds to the T cell receptor/CD3 complex [44] activating lymphocytes largely via phospholipase C-mediated events [45]. Subsequently, the second messengers inositol-1,4,5-trisphosphate and diacylglycerol are generated, and then intracellular calcium concentrations become elevated and protein kinase C becomes activated [45]. FO feeding has been shown to inhibit the activation of phospholipase C-γ1 in rat lymphocytes with a concomitant decrease in inositol-1,4,5-trisphosphate generation [46] and to decrease the generation of diacylglycerol and ceramide in Con A-stimulated mouse lymphocytes [32]. These actions would serve to diminish the elevation in intracellular free calcium concentrations and the activation of protein kinase C, thereby having the general effect of decreasing cellular responses. In addition, eicosapentaenoic and docosahexaenoic acids can directly inhibit the activity of spleen lymphocyte protein kinase C [47]. Thus, there are several potential early sites at which the inhibitory effects of the components of FO could be exerted.

A FO-induced shift away from a Th1-type response may explain the low incidence of inflammatory and autoimmune disorders among Greenland Eskimos [3]. Furthermore, it could explain some of the benefits that have been observed following the administration of FO to patients with rheumatoid arthritis [see refs. 48, 49 for reviews], ulcerative colitis [see 50 for a review], Crohn's disease [see 51 for a review], and psoriasis [see 52 for a review]. FO also prolongs the survival of cardiac transplants in rats [53–55], and renal transplants survive and/or function better in patients who receive FO [56–59] and this effect might be due to a shift away from a Th1-type response.

In summary, the type of fatty acid in the diet can modulate lymphocyte proliferation and the production of cytokines by lymphocytes. Th1-type cytokines are more sensitive to the effects of fatty acids than are Th2-type cytokines and the long-chain *n*-3 PUFAs, as found in FO, appear to be the most potent fatty acids in this regard. The effects of fatty acids on cytokine production appear to be exerted through effects on cytokine gene expression.

ACKNOWLEDGMENTS

This work was supported by grant number 51/FO5696 from the BBSRC. F. A. W. held a Rank Prize Fund Studentship when this work was performed.

REFERENCES

1. Roper, R. L., Phipps, R. P. (1994) Prostaglandin E_2 regulation of the immune response. Adv. Prost. Thromb. Leuk. Res. 22, 101–111.
2. Roper, R. L., Brown, D. M., Phipps, R. P. (1995) Prostaglandin E_2 promotes B lymphocyte Ig isotype switching to IgE. J. Immunol. 154, 162–170.
3. Kromann, N., Green, A. (1980) Epidemiological Studies in the Upernavik District, Greenland. Acta Med. Scand. 208, 401–406.
4. Tsang, W. M., Weyman, C., Smith, A. D. (1977) Effect of fatty acid mixtures on phytohaem-agglutinin-stimulated lymphocytes of different species. Biochem. Soc. Trans. 5, 153–154.
5. Offner, H., Clausen, J. (1974) Inhibition of lymphocyte response to stimulants induced by unsaturated fatty acids and prostaglandins in multiple sclerosis. Lancet 2, 1204–1205.
6. Mertin, J., Hughes, D. (1975) Specific inhibitory action of polyunsaturated fatty acids on lymphocyte transformation induced by PHA and PPD. Int. Arch. Allergy Appl. Immunol. 48, 203–210.
7. Weyman, C., Belin, J., Smith, A. D., Thompson, R. H. S. (1975) Linoleic acid as an immunosuppressive agent. Lancet 2, 33.
8. Calder, P. C., Bond, J. A., Bevan, S. J., Hunt, S. V., Newsholme, E. A. (1991) Effect of fatty acids on the proliferation of concanavalin A-stimulated rat lymph node lymphocytes. Int. J. Biochem. 23, 579–588.
9. Calder, P. C., Newsholme, E. A. (1992) Unsaturated fatty acids suppress interleukin-2 production and transferrin receptor expression by concanavalin A-stimulated rat lymphocytes. Mediat. Inflamm. 1, 107–115.
10. Brouard, C., Pascaud, M. (1993) Modulation of rat and human lymphocyte function by *n*-6 and *n*-3 polyunsaturated fatty acids and acetylsalicylic acid. Ann. Nutr. Metab. 37, 146–159.
11. Santoli, D., Phillips, P. D., Colt, T. L., Zurier, R. B. (1990) Suppression of interleukin-2-dependent human T cell growth in vitro by prostaglandin E (PGE) and their precursor fatty acids. J. Clin. Invest. 85, 424–432.
12. Calder, P. C., Newsholme, E. A. (1992) Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2 production. Clin. Sci. 82, 695–700.
13. Soyland, E., Nenseter, M. S., Braathen, L., Drevon, C. A. (1993) Very long chain *n*-3 and *n*-6 polyunsaturated fatty acids inhibit proliferation of human T lymphocytes in vitro. Eur. J. Clin. Invest. 23, 112–121.
14. Purasiri, P., McKechnie, A., Heys, S. D., Eremin, O. (1997) Modulation in vitro of human natural cytotoxicity, lymphocyte proliferative response to mitogens and cytokine production by essential fatty acids. Immunology 92, 166–172.
15. Khalfoun, B., Thibault, G., Lacord, M., Gruel, Y., Bardos, P., Lebranchu, Y. (1996) Docosahexaenoic and eicosapentaenoic acids inhibit human lymphoproliferative responses in vitro but not the expression of T cell surface activation markers. Scand. J. Immunol. 43, 248–256.
16. Kollmorgen, G. M., Sansing, W. A., Lehman, A. A., Fischer, G., Langley, R. E., Alexander, S. S., King, M. M., McCay, P. B. (1979) Inhibition of lymphocyte function in rats fed high-fat diets. Cancer Res. 39, 3458–3642.
17. Locniskar, M., Nauss, K. M., Newberne, P. M. (1983) The effect of quality and quantity of dietary fat on the immune system. J. Nutr. 113, 951–961.
18. Alexander, N. J., Smythe, N. L. (1988) Dietary modulation of in vitro lymphocyte function. Ann. Nutr. Metab. 32, 192–199.
19. Friend, J. V., Lock, S. O., Gurr, M. I., Parish, W. E. (1980) Effect of different dietary lipids on the immune responses of Hartley strain guinea pigs. Int. Arch. Allergy Appl. Immunol. 62, 292–301.
20. Levy, J. A., Ibrahim, A. B., Shirai, I., Okta, K., Nagasawa, R., Yoshida, H., Estes, J., Gardner, M. (1982) Dietary fat affects immune response, production of antiviral factors and immune complex disease in NZB/NZW mice. Proc. Natl. Acad. Sci. USA 79, 1974–1978.
21. Morrow, W. J. W., Ohashi, Y., Hall, J., Pribnow, J., Hirose, S., Shirai, T., Levy, J. A. (1985) Dietary fat and immune function. I. Antibody responses, lymphocyte and accessory cell function in (NZB x NZW)F1 mice. J. Immunol. 135, 3857–3863.
22. Yaqoob, P., Newsholme, E. A., Calder, P. C. (1994) The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. Immunology 82, 603–610.
23. Ossman, J. B., Erickson, K. L., Cantoly, N. L. (1980) Effects of saturation and concentration of dietary fats on lymphocyte transformation in mice. Nutr. Rep. Int. 22, 279–284.
24. Yaqoob, P., Calder, P. C. (1995) The effects of dietary lipid manipulation on the production of murine T-cell-derived cytokines. Cytokine 7, 548–553.
25. Erickson, K. L., McNeill, C. J., Gershwin, M. E., Ossman, J. B. (1980) Influence of dietary fat concentration and saturation on immune ontogeny in mice. J. Nutr. 110, 1555–1572.

26. Marshall, L. A., Johnston, P. V. (1985) The influence of dietary essential fatty acids on rat immunocompetent cell prostaglandin synthesis and mitogen-induced blastogenesis. J. Nutr. 115, 1572–1580.
27. Jeffery, N. M., Sanderson, P., Sherrington, E. J., Newsholme, E. A., Calder, P. C. (1996) The ratio of *n*-6 to *n*-3 polyunsaturated fatty acids in the rat diet alters serum lipid levels and lymphocyte functions. Lipids 31, 737–745.
28. Kelley, D. S., Branch, L. B., Love, J. E., Taylor, P. C., Rivera, Y. M., Iacono, J. M. (1991) Dietary alpha-linolenic acid and immunocompetence in humans. Am. J. Clin. Nutr. 53, 40–46.
29. Meydani, S. N., Endres, S., Woods, M. M. Goldin, B. R., Soo, C., Morrill-Labrode, A., Dinarello, C., Gorbach, S. L. (1991) Oral (*n*-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. J. Nutr. 121, 547–555.
30. Meydani, S. N., Lichtenstein, A. H., Cornwall, S., Meydani, M., Goldin, B. R., Rasmussen, H., Dinarello, C. A., Schaefer, E. J. (1993) Immunologic effects of national cholesterol education panel step-2 diets with and without fish-derived *n*-3 fatty acid enrichement. J. Clin. Invest. 92, 105–113.
31. Turek, J. J., Schoenlein, I. A., Clark, L. K., van Alstine, W. G. (1994) Dietary polyunsaturated fatty acids effects on immune cells of the porcine lung. J. Leukoc. Biol. 56, 599–604.
32. Jolly, C. A., Jiang, Y-H., Chapkin, R. S., McMurray, D. N. (1997) Dietary (*n*-3) polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion and the formation of diacylglycerol and ceramide. J. Nutr. 127, 37–43.
33. Endres, S., Meydani, S. N., Ghorbani, R., Schindler, R., Dinarello, C. A. (1993) Dietary supplementation with *n*-3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. J. Leukoc. Biol. 54, 599–603.
34. Gallai, V., Sarchielli, P., Trequattrini, A., Franceschini, M., Floridi, A., Firenze, C., Alberti, A., Di Benedetto, D., Stragliotto, E. (1993) Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with *n*-3 polyunsaturated fatty acids. J. Neuroimmunol. 56, 143–153.
35. Mossman, T. T., Sad, S. (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol. Today 17, 138–146.
36. Romagnani, S. (1997) The Th1/Th2 paradigm. Immunol. Today 18, 263–266.
37. Folch, J., Lees, M., Stanley, G. H. S. (1957) A simple method for the isolation and purification of lipides from animal tissues. J. Biol. Chem. 226, 497–509.
38. Yaqoob, P., Newsholme, E. A., Calder, P. C. (1995) Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. Biochim. Biophys. Acta 1255, 333–340.
39. Gibney, M. J., Hunter, B. (1993) The effects of short- and long-term supplementation with fish oil on the incorporation of *n*-3 polyunsaturated fatty acids into cells of the immune system in healthy volunteers. Eur. J. Clin. Nutr. 47, 255–259.
40. Yaqoob, P., Pala, H. S., Cortina-Borja, M., Newsholme, E. A., Calder, P. C. (2000) Encapsulated fish oil enriched in α-tocopherol alters plasma phospholipid and mononuclear cell fatty acid compositions but not mononuclear cell functions. Eur. J. Clin. Invest. 30, 260–274.
41. Smith, K. A. (1988) Interleukin-2: Inception, impact and implications. Science 240, 1169–1176.
42. Jolly, C. A., McMurray, D. N., Chapkin, R. S. (1998) Effect of dietary *n*-3 fatty acids on interleukin-2 and interleukin-2 receptor α expression in activated murine lymphocytes. Prost. Leuk. Ess. Fatty Acids 58, 289–293.
43. Kleemann, R., Scott, F. W., Worz-Pagenstert, U., Ratnayake, W. M. N., Kolb, H. (1998) Impact of dietary fat on Th1/Th2 cytokine gene expression in the pancreas and gut of diabetes-prone BB rats. J. Autoimmun. 11, 97–103.
44. Licastro, F., Davis, L. J., Morini, M. C. (1993) Lectins and superantigens—membrane interactions of these compounds with T-lymphocytes affect immune responses. Int. J. Biochem. 25, 845–852.
45. Berry, N., Nishizuka, Y. (1990) Protein kinase C and T-cell activation. Eur. J. Biochem. 189, 205–214.
46. Sanderson, P., Calder, P. C. (1998) Dietary fish oil appears to inhibit the activation of phospholipase C-γ in lymphocytes. Biochim. Biophys. Acta 1392, 300–308.
47. May, C. L., Southworth, A. J., Calder, P. C. (1993) Inhibition of lymphocyte protein kinase C by unsaturated fatty acids. Biochem. Biophys. Res. Commun. 195, 823–828.
48. James, M. J., Cleland, L. G. (1997) Dietary *n*-3 fatty acids and therapy for rheumatoid arthritis. Semin. Arth. Rheum. 27, 85–97.
49. Geusens, P. P. (1998) *n*-3 Fatty acids in the treatment of rheumatoid arthritis. In *Medicinal Fatty Acids in Inflammation* (J. Kremer, ed.), Basel: Birkhauser Verlag, 111–123.
50. Rodgers, J. B. (1998) *n*-3 Fatty acids in the treatment of ulcerative colitis. In *Medicinal Fatty Acids in Inflammation* (J. Kremer, ed.), Basel: Birkhauser Verlag, 103–109.
51. Belluzzi, A., Miglio, F. (1998) *n*-3 Fatty acids in the treatment of Crohn's disease. In *Medicinal Fatty acids in Inflammation* (J. Kremer, ed.), Basel: Birkhauser Verlag, 91–101.
52. Ziboh, V. A. (1998) The role of *n*-3 fatty acids in psoriasis. In *Medicinal Fatty Acids in Inflammation* (J. Kremer, ed.), Basel: Birkhauser Verlag, 45–53.
53. Otto, D. A., Kahn, D. R., Hamm, M. W., Forrest, D. E., Wooten, J. T. (1990) Improved survival of heterotopic cardiac allografts in rats with dietary *n*-3 polyunsaturated fatty acids. Transplantation 50, 193–198.
54. Grimm, H., Tibell, A., Norrlind, B., Schott, J., Bohle, R. A. (1995) Nutrition and allorejection impact of lipids. Transplant. Immunol. 3, 62–67.
55. Grimminger, F., Grimm, H., Fuhrer, D., Papavassilis, C., Lindemann, G., Blecher, C., Mayer, K., Tabesch, F., Kramer, H. J., Stevens, J., Seeger, W. (1996) Omega 3 lipid infusion in a heart allotransplant model: shift in fatty acid and lipid mediator profiles and prolongation of transplant survival. Circulation 93, 365–371.
56. Berthoux, F. C., Guerin, C., Berthoux, P., Burgard, G., Alamartine, E. (1992) One-year randomized controlled trial with omega-3 fatty acid-rich fish oil in clinical renal transplant. Transplant. Proc. 24, 2578–2582.
57. van der Heide, H. J. J., Bilo, H. J. G., Donker, J. M., Wilmink, J. M., Tegzess, A. M. (1993) Effect of dietary fish oil on renal function and rejection in cyclosporine-treated recipients of renal transplants. N. Engl. J. Med. 329, 769–773.
58. Bennett, W. M., Carpenter, C. B., Shapiro, M. E., Strom, T. B., Hefty, D., Tillman, M., Abrams, J., Ryan, D., Kelley, V. A. (1995) Delayed omega-3 fatty acid supplements in renal transplantation. Transplantation 59, 352–356.
59. Maachi, K., Berthoux, P., Burgard, G., Alamartine, E., Berthoux, F. (1995) Results of a 1-year randomized controlled trial with omega-3 fatty acid fish oil in renal transplantation under triple immunosuppressive therapy. Transplant. Proc. 27, 846–849.