Monounsaturated fats and immune function

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The Mediterranean diet has become a cultural model for dietary improvement. Since the 1950s there has been growing evidence that Mediterranean countries display rates of chronic diseases that are amongst the lowest in the world and life expectancies that are amongst the highest (Nestle, 1995). The Mediterranean diet is characterized by the use of olive oil as the major culinary fat and, although the total intake of fat may be relatively high, this is strongly correlated with a low intake of saturated fat (Keys, 1970). As the Seven Countries Study clearly showed, it is the type of fat rather than the level of fat consumed in the diet that is most closely related to the incidence of CHD (Keys, 1970) and subsequent studies have shown that the replacement of saturated fatty acids (SFA) with either monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) may be beneficial (Mensink & Katan, 1987; Mata *et al.* 1992).

Since the consumption of diets rich in MUFA has been linked with a low prevalence of atherosclerosis, there has been great interest in the effects of MUFA on lipoprotein metabolism. Less attention has been paid to the effects of MUFA on the immune system, yet cells of the immune system are an inherent part of the inflammatory events which are involved in the development and progression of atherosclerosis.

Olive oil has classically been used as a placebo treatment in studies investigating the effects of fish oils on immune function and in various human disease conditions, since MUFA have typically been regarded as being neutral (Cleland *et al.* 1988; Virella *et al.* 1991). However, a number of clinical trials have reported effects of the olive oil treatment which are equal or similar to the effects of the treatment under test (usually fish oil). One such study reported no significant difference between fish oil supplements and an olive oil placebo in preventing restenosis after coronary angioplasty (Dehmer *et al.* 1988); a subsequent letter to *Lancet* suggests that '. . . future studies of oil supplements should not consider olive oil as a placebo until there are more data evaluating the role of MUFA . . .' (Milner, 1989).

A smaller number of studies have suggested that there may be beneficial effects of olive oil consumption on rheumatoid arthritis (Kremer *et al.* 1990), an autoimmune disease characterized by infiltration of synovial tissues and fluid by cells of the immune system and vigorous overactivity and inflammation therein. In particular, a much-cited study by Linos *et al.* (1991) shows that frequent consumption of olive oil decreases the relative risk for developing rheumatoid arthritis. It is proposed that the suppressive effect of olive oil on the development of rheumatoid arthritis may be exerted via an effect on the immune system.

Studies investigating the effects of MUFA-rich diets on immune functions have been overshadowed often by those which investigate the feeding of diets rich in *n*-6 (such as maize, soyabean, safflower or sunflower oils) or *n*-3 PUFA (such as linseed or fish oils). In general, the *n*-6 PUFA are believed to enhance immune function and the *n*-3 PUFA to suppress it (Yaqoob & Calder, 1993). However, there is now growing evidence that MUFA-rich oils, which were previously thought to be neutral with respect to immune function, have effects which are similar to those of fish oils. The purpose of the present review is to discuss and evaluate the evidence that monounsaturated fats can influence the composition and functions of cells of the immune system.

Effects of olive oil on *ex vivo* lymphocyte proliferation

The *in vitro* effects of fatty acids on lymphocyte proliferation have been studied since the early 1970s and have been reviewed in detail elsewhere (Gurr, 1983; Yaqoob & Calder, 1993). These studies have investigated the effects of a large range of fatty acids, including oleic acid, the major fatty acid contained in olive oil, but the results are disparate and comparisons between studies are made difficult by the differences in the concentrations of fatty acids used, the cell type studied, the means by which they were presented to cells and the conditions of incubation. The *in vitro* studies therefore remain contradictory, some showing no effect of oleic acid and some showing a suppression of lymphocyte proliferation (Gurr, 1983; Yaqoob & Calder, 1993).

In order to obtain information about the effects of dietary lipids, we have investigated the effects of feeding rats a range of high-fat (200 g/kg) diets, each with a characteristic fatty acid composition, or a low-fat (25 g maize oil/kg) diet on lymphocyte fatty acid composition and on a number of lymphocyte functions. In these studies, the animals were fed on hydrogenated coconut oil, olive oil, safflower oil, evening primrose (*Oenothera biennis*) oil or fish (menhaden) oil for

Abbreviations: Con A, concanavalin A; DHA, docosahexaenoic acid; G *v.* H, graft *v.* host; H *v.* G, host *v.* graft; HSVEC, human saphenous-vein endothelial cells; ICAM-1, intercellular adhesion molecule-1; MUFA, monounsaturated fatty acids; NK, natural killer; PBMNC, peripheral-blood mononuclear cells; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TNFα, tumour necrosis factor-α; VCAM-1, vascular-cell adhesion molecule-1.

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a period of 10 weeks. The first of these studies reported a significant suppressive effect of olive oil on the *ex vivo* proliferation of mesenteric lymph node lymphocytes in response to the T-cell mitogen, concanavalin A (Con A), when compared with feeding a low-fat diet or diets rich in *hydrogenated* coconut oil or safflower oil (Yaqoob *et al.* 1994*a*; see Fig. 1). The effect of the olive oil diet was similar in magnitude to those of the fish oil or evening primrose oil diets (Fig. 1) and was also demonstrated in whole-blood cultures stimulated with suboptimal concentrations of Con A (Yaqoob *et al.* 1995*a*; see Fig. 2). All the high-fat diets were shown to modulate the fatty acid composition of lymphocytes, resulting in characteristic profiles for each dietary group (Yaqoob *et al.* 1995*b*). Only one other study has examined the effects of feeding an olive oil-rich diet on lymphocyte proliferation in rodents. The study of Berger *et al.* (1993) compared the effects of feeding a low-fat (30 g fat/kg), olive oil (100 g/kg), safflower oil (100 g/kg), linseed oil (100 g/kg) or fish plus safflower oil (90 plus 10 g/kg respectively) diet to dams for 5 months on the proliferation of Con A-stimulated murine spleen lymphocytes of their pups before weaning. In contrast to the studies described previously, they found no effect of dietary manipulation (Berger *et al.* 1993). There are several possible reasons for this finding. First, the high-fat diets used by Berger *et al.* (1993) contained half the total amount of fat used in our studies. Second, Berger *et al.* (1993) fed murine dams on each of the diets and then tested lymphocyte proliferation using cells from the pups before weaning, whereas our studies used rats fed for 10 weeks immediately after weaning. It is possible that in the study of Berger *et al.* (1993) the sucking pups may not have been exposed to milk of sufficiently differing fatty acid composition to allow dietary lipid manipulation to occur through this transition. Third, in the study by Berger *et al.* (1993) lymphocytes were cultured in fetal calf serum, whereas in our studies cells were cultured in autologous serum or as whole-blood cultures; we have shown that culturing cells for 48 h in fetal calf serum, but not autologous serum, reverses the changes in fatty acid composition brought about by dietary lipid manipulation (Yaqoob *et al.* 1995*b*) and negates any effects of dietary lipid manipulation on cell function (Yaqoob *et al.* 1994*a*; Fig. 1).

Since olive oil contains a number of antioxidants, sterols, hydrocarbons and alcohols (Gunstone *et al.* 1994), it seemed important to determine whether the effects observed following feeding of this diet to rats were due to oleic acid or to some other component of the oil. This was investigated using a diet rich in high-oleic sunflower oil (Jeffery *et al.* 1996); in earlier studies, feeding the safflower oil diet had no effect on proliferation of lymph node lymphocytes (Yaqoob *et al.* 1994*a*; Fig. 1) or of whole-blood cultures (Yaqoob *et al.* 1995*a*; Fig. 2). The effects of feeding a diet containing high-oleic sunflower oil, therefore, were compared with the effects of feeding the low-fat, olive oil or safflower oil diets (Jeffery *et al.* 1996). The fatty acid composition of spleen lymphocyte lipids was strongly affected by that of the diets fed; importantly, the high-oleic sunflower oil and olive oil diets resulted in a significantly higher proportion of oleic acid in lymphocyte lipids than the low-fat or safflower oil diets (Jeffery *et al.* 1996). Feeding either the olive oil or the high-oleic sunflower oil diet significantly decreased the proliferation of spleen lymphocytes compared with feeding the low-fat or safflower oil diets; the effects of the olive oil and

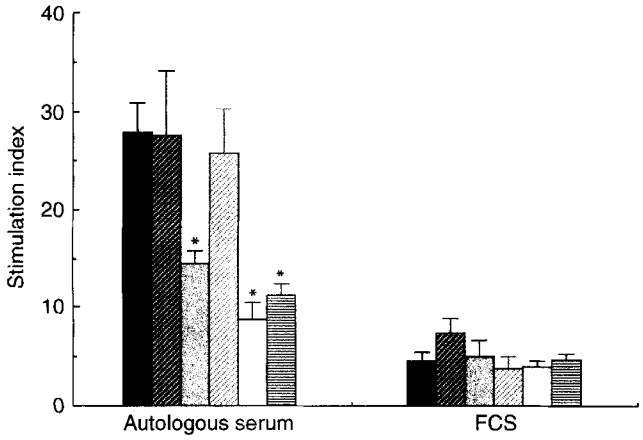

Fig. 1. Effect of dietary lipid manipulation on proliferation of rat mesenteric lymph node lymphocytes cultured in autologous serum or fetal calf serum (FCS). Rats were fed for 10 weeks on either a low-fat diet (▬) or diets containing 200 g coconut oil (▨), olive oil (▩), safflower oil (▨), evening primrose (*Oenothera biennis*) oil (▭) or fish oil (▤)/kg. Proliferation in response to 5 μg concanavalin A/ml was measured by incorporation of [^{3}H]thymidine and expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the low-fat diet (ANOVA): * $P < 0{\cdot}05$. (Data taken from Yaqoob *et al.* 1994*a*.)

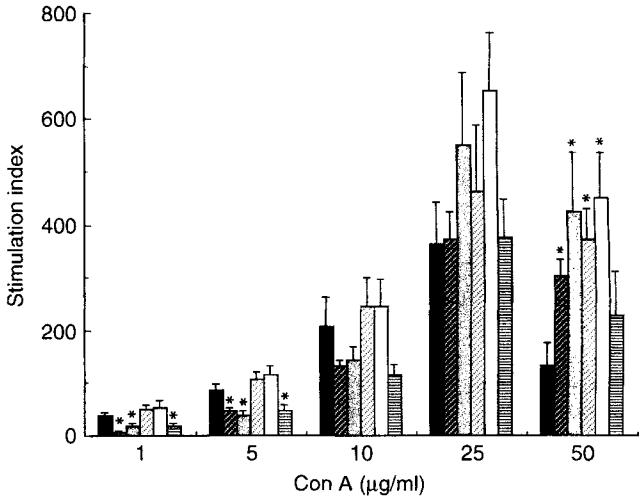

Fig. 2. The effect of dietary lipid manipulation on the proliferation of concanavalin A (Con A)-stimulated whole-blood cultures. Rats were fed for 10 weeks on either a low-fat diet (▬) or diets containing 200 g coconut oil (▨), olive oil (▩), safflower oil (▨), evening primrose (*Oenothera biennis*) oil (▭) or fish oil (▤)/kg. Proliferation of whole blood (heparinized blood diluted 1 : 10 (v/v) with Roswell Park Memorial Institute culture medium containing 2 mM-glutamine and antibiotics) in response to Con A was measured by incorporation of [^{3}H]thymidine and expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those for the low-fat diet (ANOVA): * $P < 0{\cdot}05$. (Data taken from Yaqoob *et al.* 1995*a*.)

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high-oleic sunflower oil diets were not significantly different from one another (Fig. 3). This suggests that the effects of the olive oil diet are likely to be due to oleic acid rather than other components of olive oil.

The studies outlined previously have used relatively large amounts of a single oil and as such they represent very extreme diets, which are unlikely to be encountered by free-living human subjects. Furthermore, the use of such oils inevitably results in variation in the levels of several fatty acids together and not only the one under investigation. A further study, therefore, investigated the effects of relatively small changes in the levels of commonly-consumed fatty acids in a controlled manner in which one fatty acid was exchanged for another, without altering the levels of other fatty acids in the diet (Jeffery *et al.* 1997). The nine diets used in this study contained 178 g fat/kg, and differed in their proportions of palmitic, oleic, linoleic and α-linolenic acids whilst maintaining a constant *n*-6 PUFA : *n*-3 PUFA value of 7 (Table 1). The effect on lymphocyte proliferation of replacing one fatty acid with another appeared to be influenced by the level of other fatty acids in the diet. On further examination, there was a significant inverse linear relationship between proliferation (expressed as stimulation index; $\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}$) and the oleic acid : linoleic acid value of the diet (r 0·331; $P = 0{\cdot}028$). However, the best-fit relationship was of the form:

$$\text{stimulation index} = a_0 + b_0 \log(\text{oleic acid : linoleic acid}),$$

where a_0 is 75·8 and b_0 is −18·56 ($P < 0{\cdot}05$ for both values). This relationship is illustrated in Fig. 4; the authors showed that lymphocyte proliferation is decreased with increasing dietary oleic acid levels up to an oleic acid level of 35·6 g/100 g fatty acids (diet E), but increasing the oleic acid level above this does not result in any further increase (Jeffery *et al.* 1997).

Studies carried out in our laboratory have recently been extended to human subjects; we have performed a study to investigate the effects of consumption of a MUFA-rich diet for 2 months on immune function in healthy, middle-aged men. Middle-aged men (mean age 48 years; range 41–56 years; BMI range 21·9–30·7 kg/m^2) were randomly assigned to consume either a control diet (designed to reproduce the current UK diet fatty acid composition) or a diet containing foods enriched with highly-refined olive oil for 8 weeks. Foods provided for subjects included the main meal of the day (as a frozen recipe meal), cooking oils and spreads, biscuits and puddings. Subjects on the MUFA diet consumed significantly less saturated fat (% energy) compared with those on the control diet and significantly more MUFA; MUFA contributed 18·4 % energy in this group compared with 11·3 % in the control group. Consumption of a MUFA-rich diet did not affect the proliferative response of either whole-blood cultures (Fig. 5) or peripheral blood

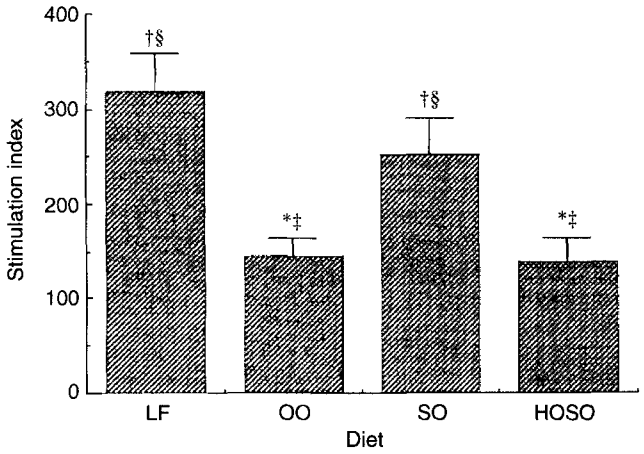

Fig. 3. The effect of feeding a high-oleic sunflower oil (HOSO) diet on proliferation of rat spleen lymphocytes. Lymphocytes were cultured in autologous serum in the absence or presence of concanavalin A; lymphocyte proliferation is expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Values are means with their standard errors represented by vertical bars. Mean values were significantly different ($P < 0{\cdot}05$ at least) from those for the following diets: * *v.* low-fat (LF), † *v.* olive oil (OO), ‡ *v.* safflower oil (SO), § *v.* HOSO. (Data taken from Jeffery *et al.* 1996.)

Table 1. Fatty acid composition of diets used in the study of Jeffery *et al.* (1997)

	Fatty acid (g/100 g total fatty acids)			
Diet	16 : 0	18 : 1*n*-9	18 : 2*n*-6	18 : 3*n*-3
A	56·6	18·5	15·4	2·1
B	37·9	19·8	30·9	4·4
C	21·9	19·7	45·8	6·3
D	10·3	25·2	51·7	6·8
E	6·2	35·6	46·2	6·6
F	22·4	36·8	30·3	4·2
G	22·4	53·1	15·6	2·2
H	5·2	53·7	30·9	4·4
I	4·5	71·6	15·4	2·2

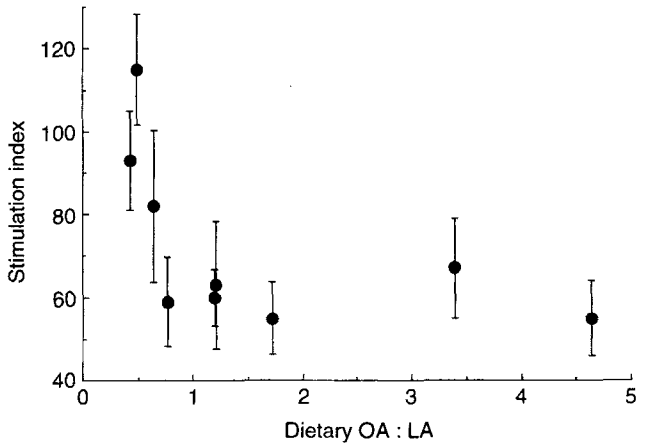

Fig. 4. Relationship between the oleic acid : linoleic acid (OA : LA) in the diet and spleen lymphocyte proliferation in rats. Lymphocytes were cultured in autologous serum in the absence or presence of concanavalin A; lymphocyte proliferation is expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Points are means with their standard errors represented by vertical bars. (Data taken from Jeffery *et al.* 1997.)

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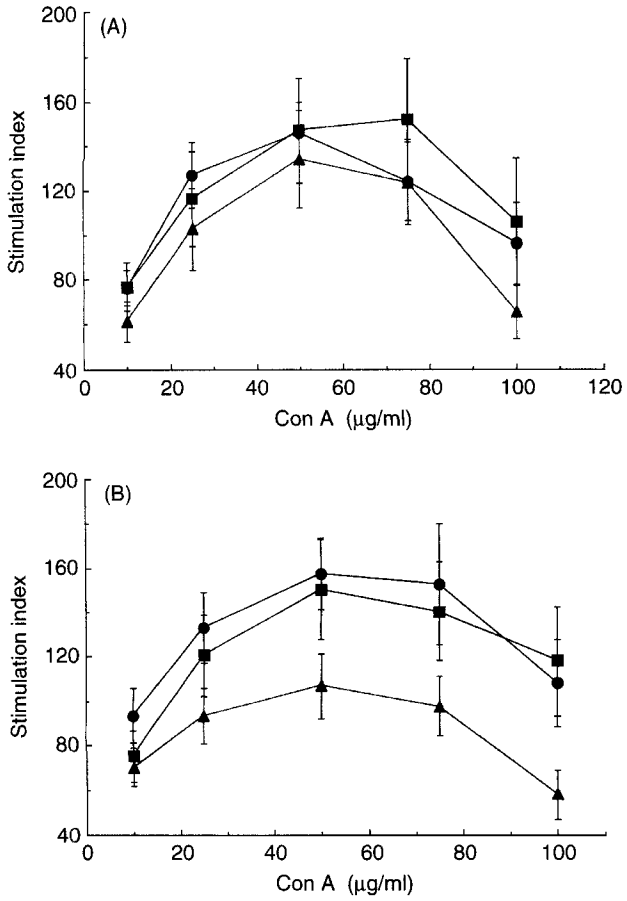

Fig. 5. Effect of monounsaturated fatty acid (MUFA) consumption on proliferation of leucocytes in whole blood in healthy middle-aged men. (A) Control group; (B) MUFA group. (■), Baseline; (●), 1 month; (▲), 2 months. Proliferation, measured as [^{3}H]thymidine incorporation, is expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Points are means with their standard errors represented by vertical bars for twenty-two to twenty-nine subjects in each group. There were no significant effects of the MUFA diet as analysed by two-way repeat-measures ANOVA. (Data taken from Yaqoob *et al.* 1998.) Con A, concanavalin A.

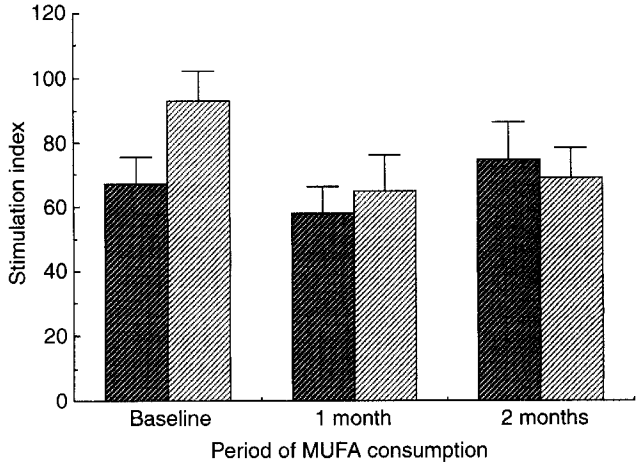

Fig. 6. Effect of monounsaturated fatty acid (MUFA) consumption on proliferation of peripheral-blood mononuclear cells in healthy middle-aged men. (▨), Control; (▨), MUFA. Proliferation, in response to 15 μg concanavalin A/ml was assessed as [^{3}H] thymidine incorporation and is expressed as stimulation index $\left(\frac{\text{incorporation of [}^3\text{H]thymidine by stimulated cells}}{\text{incorporation of [}^3\text{H]thymidine by unstimulated cells}}\right)$. Values are means with their standard errors for twenty-one to twenty-nine subjects in each group. There were no significant differences in the effects of time and diet and their interactions as analysed by two-way repeat-measures ANOVA. (Data taken from Yaqoob *et al.* 1998.)

mononuclear cells (PBMNC; Fig. 6) to the T-cell mitogen, Con A (Yaqoob *et al.* 1998). This observation contrasts with results obtained using laboratory animals (Yaqoob *et al.* 1994*a*, 1995*a*). The effect of the MUFA diet may well have been significant if the study had been longer, particularly if the fatty acid composition of plasma and cells had continued to change; it is not clear whether the changes in fatty acid composition had reached a plateau within the time period studied. However, the lack of a clear effect of MUFA may be attributable to the higher level of monounsaturated fat used in the animal studies; in these studies rats were fed for 10 weeks on diets containing 200 g olive oil/kg (MUFA therefore contributed approximately 30 % of total energy intake), whereas in the human study, MUFA contributed approximately 18 % of the total energy intake. While it is possible that a higher level of dietary MUFA may have resulted in suppression of proliferation, the purpose of the study was to examine the effects of intakes which are in no way extreme; the intakes employed in the human study closely correspond to current Mediterranean intakes (Ferro-Luzzi & Branca, 1995) and can readily be achieved through consumption of meals which use olive oil as the primary cooking fat.

Effects of olive oil on *ex vivo* natural killer cell activity

One of the most important mechanisms by which the immune system deals with foreign cells is to damage or destroy them. Typical target cells include malignant cells, normal cells of the host that are infected with viruses or other micro-organisms and normal cells from individuals unrelated to the responding host. Natural killer (NK) cells are a subset of lymphocytes found mainly in blood and the spleen (Herberman, 1988). They are derived from the bone marrow, but are neither T-cells nor B-cells and they do not undergo thymic maturation. Killing by NK cells is part of natural rather than specific immunity, since it is not induced by a specific antigen and is not restricted by major histocompatibility complex molecules.

Feeding rats for 10 weeks on a diet containing 200 g olive oil/kg results in significant suppression of NK cell activity compared with feeding a low-fat diet or diets containing 200 g hydrogenated coconut oil or safflower oil/kg (Yaqoob *et al.* 1994*b*; Table 2). The inhibition of NK activity is greater than that produced by feeding a diet rich in evening primrose oil, but not as great as that resulting from feeding a diet containing 200 g fish oil/kg (Table 2). As with the experiments on lymphocyte proliferation described previously, when the effects of high-oleic sunflower oil were compared with those of olive oil, NK cell activity was significantly lower for spleen lymphocytes from rats fed on the olive oil or the

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Table 2. The effect of dietary lipid manipulation on natural killer activity in freshly-prepared rat spleen lymphocytes (Data taken from Yaqoob *et al.* 1994*b*)

(Cytolysis of YAC-1 (target) cells by rat spleen lymphocytes (effector cells) was measured by release of ^{51}Cr by pre-loaded YAC-1 cells at various values for effector : target cells (E : T). Results are expressed as % cytolysis. Values are means with their standard errors)

	% Cytolysis							
E : T...	100 : 1		50 : 1		25 : 1		12·5 : 1	
Diet	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
LF	46·5^{cef}	2·0	29·7^{cef}	2·8	17·7^{bcdef}	2·1	7·4^{cef}	1·2
HCO	40·4^{f}	2·4	22·9^{f}	1·5	11·5^{afe}	0·6	4·5	0·8
OO	36·4^{a}	2·8	20·8^{af}	1·7	9·3^{af}	0·9	3·4^{a}	0·4
SO	42·1^{f}	1·5	24·2^{f}	1·3	11·2^{af}	1·1	3·9	0·7
EPO	40·7^{af}	0·7	19·1^{a}	2·6	8·9^{abf}	0·2	3·2^{a}	0·4
MO	29·4^{abde}	2·1	13·6^{abcd}	0·7	5·0^{abcde}	0·3	3·1^{a}	0·8

LF, low-fat; HCO, hydrogenated coconut oil; OO, olive oil; SO, safflower oil, EPO, evening primrose (*Oenothera biennis*) oil; MO, menhaden oil.
^{a,b,c,d,e,f} Mean values were significantly different ($P < 0·05$) for the following comparisons: ^{a} *v.* LF, ^{b} *v.* HCO, ^{c} *v.* OO, ^{d} *v.* SO, ^{e} *v.* EPO, ^{f} *v.* MO.

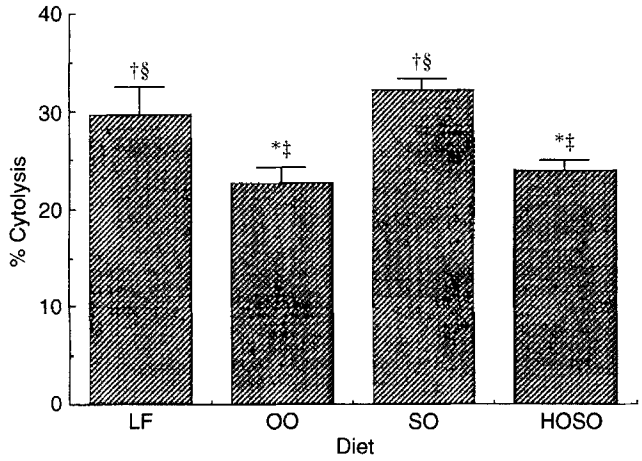

Fig. 7. The effect of feeding a high-oleic sunflower oil (HOSO) diet on rat spleen lymphocyte natural killer cell activity. Cytolysis of YAC-1 (target) cells by rat spleen lymphocytes (effector cells) was measured by release of ^{51}Cr by pre-loaded YAC-1 cells at a ratio of 100 : 1 effector : target cells. Results are expressed as % cytolysis. Values are means with their standard errors represented by vertical bars. Mean values were significantly different ($P < 0·05$ at least) from those for the following diets: * *v.* low-fat (LF), † *v.* olive oil (OO), ‡ *v.* sunflower oil (SO), § *v.* HOSO. (Data taken from Jeffery *et al.* 1996.)

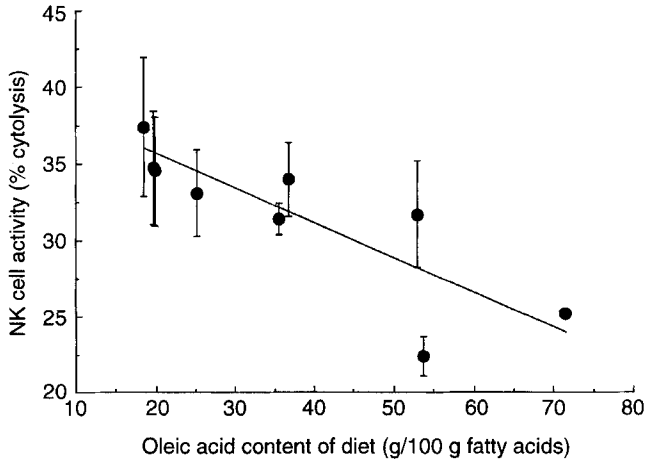

Fig. 8. Relationship between the oleic acid content of the diet and spleen natural killer (NK) cell activity in rats. Points are means with their standard errors represented by vertical bars. (From Jeffery *et al.* 1997.)

high-oleic sunflower oil than for those from rats fed on the low-fat or the safflower oil diets; the effects of the olive and high-oleic sunflower oils were not significantly different from one another (Fig. 7). Once again, this finding suggests that the effects of the olive oil diet are likely to be due to oleic acid rather than other components of olive oil.

In a study comparing the effects of diets A to I (Table 1) on NK activity, there was a significant negative linear relationship between the oleic acid content of the diet and NK cell activity, suggesting that dietary oleic acid causes diminished NK cell activity (Fig. 8). Furthermore, there was a negative relationship between oleic acid : linoleic acid in the diet and NK cell activity and a weak negative relationship ($r - 0·289$; $P = 0·092$) between NK cell activity and the level of oleic acid in spleen lymphocytes (Jeffery *et al.* 1997).

The study by Berger *et al.* (1993) also examined the effects of a range of high-fat diets on NK cell activity. This study showed no effect of an olive oil-rich diet on NK cell activity in mice when compared with a low-fat, safflower oil or linseed oil diet (Berger *et al.* 1993); once again, the reasons for the lack of effect may be attributable to the amount of fat in the diet and/or the protocol used (dams fed for 5 months and pups subsequently used before weaning).

In a study investigating the effects of MUFA on immune function in healthy, middle-aged men (Yaqoob *et al.* 1998), consumption of the MUFA diet produced a small decrease in NK cell activity at 2 months, but not at 1 month (Table 3). However, this was not statistically significant when compared either with the baseline or with the control group, largely due to the small sample size for the control group at 2 months (Table 3). NK cell activity was unaffected by consumption of the control diet (Table 3). As was seen with the effects of olive oil on lymphocyte proliferation, this observation contrasts with animal studies, which have shown a strong suppression of NK cell activity by an olive oil-rich diet (Yaqoob *et al.* 1994*b*) and may be attributable to the higher level of monounsaturated fat used in the animal studies. It is interesting to note, however, that the small changes in NK cell activity and proliferation observed after 2 months

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Table 3. Effect of monounsaturated fatty acid (MUFA) consumption on natural killer cell activity of peripheral-blood mononuclear cells (PBMNC) in healthy middle-aged men (Data taken from Yaqoob *et al.* 1998)

(Natural killer cell activity was determined by measuring release of lactate dehydrogenase (*EC* 1.1.1.2) from target cells (K562) as a result of lysis by effector cells (PBMNC). Values are means with their standard errors for the no. of analyses shown in parentheses. Two-way repeat-measures ANOVA showed no significant effect of diet or time)

	% Cytolysis											
Period on MUFA...	Baseline				1 month				2 months			
	Control (19)		MUFA (21)		Control (25)		MUFA (24)		Control (15)		MUFA (19)	
E : T	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
100 : 1	60·9	4·2	58·8	4·6	63·7	3·2	58·7	4·0	63·0	9·3	52·4	4·5
50 : 1	41·5	3·6	40·7	3·3	43·3	3·1	36·9	3·9	43·2	4·9	34·2	3·4
25 : 1	28·6	3·4	25·1	2·8	28·0	2·4	24·2	2·8	25·4	2·9	23·5	3·3
12·5 : 1	18·8	2·5	15·2	2·0	18·5	1·7	14·3	1·8	16·0	2·2	13·4	2·4

E : T, effector : target cells.

of consumption of the MUFA diet were accompanied by a small, but significant, increase in the proportion of oleic acid in plasma phospholipids and in PBMNC (Yaqoob *et al.* 1998).

Effects of olive oil on expression of adhesion molecules

Recent advances in our understanding of basic mechanisms of inflammation, of cell–cell interactions and of leucocyte trafficking have highlighted the importance of adhesive interactions between leucocytes and cellular or extracellular components of tissues. There has been a significant expansion of knowledge regarding the role of cell surface adhesion molecules in these processes over the last 10 years and a number of adhesion molecules have been classified into families according to sequence homology and functions. It has been suggested that some adhesion molecules may have pathophysiological as well as physiological roles; some adhesion molecules have been implicated in the trans-endothelial migration of leucocytes into synovial tissue and fluid in rheumatoid arthritis and in leucocyte–endothelium interactions which lead to the formation of atherosclerotic plaques (Munro, 1993). There has consequently been a great deal of interest in recent years in the potential modulation of the expression and/or functions of adhesion molecules by fatty acids, particularly those of the *n*-3 PUFA family. Once again, the intense interest in fish oils and *n*-3 PUFA has overshadowed some potentially exciting effects of MUFA on adhesion molecule expression.

In a study by De Caterina *et al.* (1994), human saphenous-vein endothelial cells (HSVEC) were pre-incubated for 24 h with arachidonic acid, docosahexaenoic acid (DHA), eicosapentaenoic acid or oleic acid (10 μM) before 6 h of stimulation with tumour necrosis factor-α (TNFα) and subsequent measurement of the surface expression of vascular-cell adhesion molecule-1 (VCAM-1). It was reported that DHA (but not eicosapentaenoic acid) and oleic acid significantly decreased the expression of VCAM-1 by HSVEC (Fig. 9). The authors concluded that since DHA produced the greatest inhibition, further experiments in the study would focus exclusively on this fatty acid. They went on to report time- and concentration-dependent inhibition of the expression of

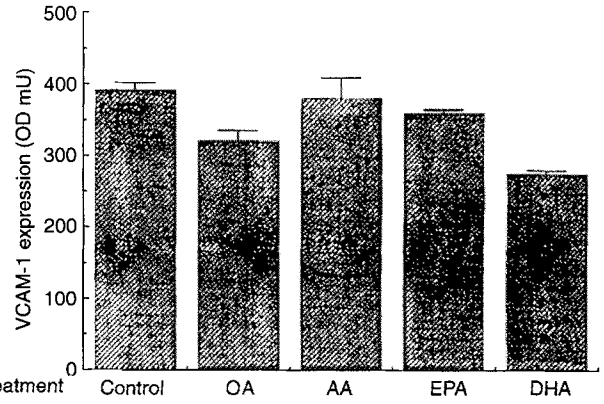

Fig. 9. Effect of pre-incubation (24 h) with fatty acids on surface expression of vascular-cell adhesion molecule (VCAM)-1 by human saphenous-vein endothelial cells in response to stimulation (6 h) with tumour necrosis factor-α. VCAM-1 expression is measured in mU optical density (OD) by a cell surface enzyme immunoassay. Expression of VCAM-1 by cells pre-incubated with oleic acid was significantly lower than control or arachidonic acid (AA)-treated cells and expression of VCAM-1 by cells pre-incubated with docosahexaenoic acid (DHA) was significantly lower than all other groups, apart from oleic acid (OA)-treated cells. EPA, eicosapentaenoic acid. (Data taken from De Caterina *et al.* 1994.)

E-selectin and intercellular adhesion molecule-1 (ICAM-1) by DHA, a reduction in the accumulation of VCAM-1 mRNA and an inhibition of the adhesion of human monocytic cells (U937) to DHA-treated HSVEC (De Caterina *et al.* 1994). Since oleic acid had the same effect on VCAM-1 expression as DHA in the initial experiment (Fig. 9), it is unfortunate that the remainder of the study focused entirely on DHA.

In a dietary study comparing the effects of a low-fat (25 g/kg) and high-fat diets containing 200 g hydrogenated coconut oil, olive oil, safflower oil, evening primrose oil or fish oil/kg, the level of expression of the adhesion molecules CD2, ICAM-1 and leucocyte-function-associated antigen-1 on rat spleen lymphocytes was decreased by both olive oil and fish oil (Sanderson *et al.* 1995*a*). In the human study carried out by our group (Yaqoob *et al.* 1998), a MUFA-rich diet resulted in a significant decrease in the expression of the

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Table 4. Effect of monounsaturated fatty acid (MUFA) consumption on expression of surface molecules on peripheral-blood mononuclear cells in healthy middle-aged men (Data taken from Yaqoob *et al.* 1998)

(Values are means with their standard errors for the no. of analyses shown in parentheses)

	% Marker positive cells												
Period on MUFA ...	Baseline				1 month				2 months				Statistical significance of difference between *groups* (ANOVA)
	Control (18–20)		MUFA (19–20)		Control (18–23)		MUFA (20–23)		Control (20–23)		MUFA (19–21)		
Surface marker	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	
ICAM-1	19·0	1·3	20·8	1·4	19·1	1·2	16·4	1·4	20·0	1·5	15·9*††	1·1	‡
Mac-1	15·5	1·2	17·0	1·8	15·4	1·4	15·4	1·3	13·7	1·3	12·6	1·5	NS§

ICAM-1, intercellular adhesion molecule-1; MAC-1, monocyte and macrophage-associated adhesion molecule.
Mean value was significantly different from that of the control group (Student's *t* test): $*P < 0·05$.
Mean value was significantly different from baseline value (Student's *t* test): $††P < 0·01$.
‡ Significant effect of diet ($P = 0·035$), and time ($P = 0·049$), with a significant interaction ($P = 0·046$).
§ No significant effect of time or diet or interaction between time and diet as analysed by two-way repeat-measures ANOVA.

leucocyte adhesion molecule, ICAM-1, after 2 months of consuming the diet compared both with baseline values and with those from the control group (Table 4). The expression of ICAM-1 did not change during the consumption of the control diet (Table 4). The MUFA diet also decreased the expression of a monocyte and macrophage-associated adhesion molecule, Mac-1, by approximately 25 % compared with baseline values. However, this decrease was not statistically significant, either compared with the baseline or with the control group (Table 4). The decrease in the expression of ICAM-1 by the MUFA diet is an important finding. ICAM-1 is a member of the immunoglobulin superfamily of adhesion molecules and is involved in leucocyte–leucocyte adhesion (Chapman & Haskard, 1995) as well as adhesion of leucocytes to endothelial cells (Munro, 1993) and to fibrinogen, a plasma adhesive protein (Languino *et al.* 1995). ICAM-1 is expressed on mononuclear cells that infiltrate inflamed synovium in patients suffering from rheumatoid arthritis (Cronstein, 1994) and in some cases, such patients may also have high levels of serum soluble ICAM-1 (Cronstein, 1994). The formation of plaques in atherosclerosis shows many features which are common to the inflammation seen in rheumatoid arthritis, such as adhesive interactions between endothelial cells and leucocytes and extravascular leucocyte accumulation and ICAM-1 is thought to play a pivotal role in the recruitment of mononuclear cells to, and therefore the growth of, the atherosclerotic plaque (Poston *et al.* 1992).

Recent evidence suggests that a MUFA-rich diet may indeed affect the process of cellular adhesion. In an interesting study by Mata *et al.* (1996), healthy men and women living in a religious community were subjected to four consecutive dietary periods (isoenergetic) differing in the fat content of SFA, MUFA, and *n*-3 and *n*-6 PUFA. It was reported that LDL-induced monocyte adhesion to endothelial cells was lower during the MUFA period than during each of the other dietary periods, and that resistance of LDL to oxidation was greatest during the MUFA period (Mata *et al.* 1996). The authors suggest that the modulation of LDL-fatty acid composition was responsible for the differences in adhesion, and showed a significant negative correlation between monocyte adhesion to endothelial cells and the oleic acid content of LDL (Mata *et al.* 1996). Expression of adhesion molecules by either cell type was not measured, but it is possible that this too may have played some role in the decreased adhesion during the MUFA period.

The fact that a high-MUFA diet appears to decrease the expression of ICAM-1 on circulating PBMNC by up to 20 % opens up the exciting possibility that the low prevalence of atherosclerosis, and perhaps other inflammatory conditions, in Mediterranean populations may at least partly involve the effects of dietary MUFA on adhesion-molecule expression; this is a potentially important area which clearly deserves further exploration.

Effects of olive oil on *in vivo* immune responses

The *ex vivo* studies described so far, whilst limited in number, build an interesting picture of the possible influences of olive oil on immune function. However, once observations have been made *ex vivo*, it is important to extend these to *in vivo* studies. Mulrooney & Grimble (1993) have achieved this by investigating the inflammatory response to TNFα in rats. This situation mimics the invasion of the body by infective and inflammatory agents, which would result in the release of cytokines from cells of the immune system. The purpose of the released cytokines, apart from modulation of the immune system, is to bring about enhanced lipolysis, gluconeogenesis, muscle proteolysis and redistribution of tissue Zn in order to provide substrates for cells of the immune system and amino acids for synthesis of acute-phase proteins. When weanling rats were fed for 8 weeks on diets containing 100 g fat/kg in the form of maize, fish, coconut oils or butter (rich in oleic acid) before an intraperitoneal injection of recombinant human TNFα, the increase in hepatic Zn concentration normally observed in the ensuing response did not occur in animals fed on the fish oil or butter diets (Mulrooney & Grimble, 1993) and the increase in plasma caeruloplasmin was smaller in the butter-fed animals than in those fed on the other diets (Mulrooney & Grimble, 1993). There was also no increase in the rate of protein synthesis in response to TNFα in the livers of animals fed on the butter diet, whereas animals fed on the maize oil and coconut oil diets demonstrated the normal increase in protein synthesis associated with the acute-phase response (Mulrooney & Grimble, 1993). In a subsequent study, it was demonstrated

that diets containing 50 or 100 g butter or olive oil/kg completely suppressed the increases in tissue Zn content, liver protein synthesis and serum caeruloplasmin levels in response to subcutaneous *Escherichia coli* endotoxin, when compared with a maize oil diet or standard laboratory chow (Besler & Grimble, 1995). In both studies, the butter and olive oil diets decreased the intensity of anorexia induced by TNFα or endotoxin (Besler & Grimble, 1995), demonstrating clearly the diminished susceptibility to the lethal effects of both agents in experimental animals.

An alternative experimental model for *in vivo* immune responses is the 'graft *v.* host' (G *v.* H) response, which can be elicited in rodents by injection of allogenic cells into the footpad of a host. The response primarily involves the polyclonal activation and proliferation of host B-cells. The 'host *v.* graft' (H *v.* G) response, on the other hand, is a T-cell-mediated response, in which cytotoxic T lymphocytes of the host recognize major histocompatibility complex antigens on the injected cells. In both cases, the enlargement of the popliteal lymph nodes (more than 15-fold in the G *v.* H response and 4-fold in the H *v.* G response) is due largely to proliferation of activated host cells within the lymph node, although there is also recruitment of cells from the bloodstream. Using this assay, Sanderson *et al.* (1995*b*) demonstrated that feeding rats a diet containing 200 g fish oil/kg suppressed the G *v.* H response compared with feeding a low-fat diet or diets containing 200 g coconut oil, safflower oil or evening primrose oil/kg; feeding a diet containing 200 g olive oil/kg had a similar effect, although the response was depressed only compared with the low-fat and evening primrose oil diets (Table 5). The expression of the adhesion molecules leucocyte-function-associated antigen-1 and ICAM-1 on lymphocytes from popliteal lymph nodes following a G *v.* H response was significantly lower in animals fed on the olive oil or fish oil diets compared with those fed on the low-fat or coconut oil diets (Sanderson *et al.* 1995*b*). It was speculated that the smaller popliteal lymph node size in animals fed on the fish oil or olive oil diets may result from a suppression of both the activation of cells within the node and of the movement of cells from the bloodstream into the nodes (Sanderson *et al.* 1995*b*). Interestingly, while the fish oil diet had a similar suppressive effect on the H *v.* G response as that on the G *v.* H response, the olive oil diet had no effect on the H *v.* G response (Table 5); it appears, therefore, that in this model, olive oil is able to modulate *in vivo* responses involving B-cells, but not those involving cytotoxic T lymphocytes. Although there are no published human studies which have set out to examine the effects of olive oil on *in vivo* immune responses, at least one study investigating the effects of fish oil supplements on immunological indices (including the systemic humoral response to tetanus toxoid) in healthy volunteers has used olive oil as a placebo treatment (Virella *et al.* 1991). The authors claim to demonstrate an immunosuppressive effect of fish oil compared with olive oil, but the protocol is far from satisfactory. Six volunteers were involved in the study, only two of whom received the olive oil treatment and, if the data are scrutinized, it is clear that a larger number of subjects may have produced different results (Virella *et al.* 1991), particularly since measurements of human immune responses are prone to substantial inter-individual variation.

Olive oil and autoimmune disorders

A much-cited study by Linos *et al.* (1991) has suggested that there may be beneficial effects of olive oil consumption on rheumatoid arthritis. This study compared the relative risk of development of rheumatoid arthritis in relation to lifelong consumption of olive oil in a Greek population, and demonstrated that high consumers of olive oil (almost every day throughout life) were four times less likely to develop rheumatoid arthritis than those who consumed olive oil less than six times per month on average throughout their lives (Linos *et al.* 1991). Interestingly, the effect of fish consumption on relative risk for rheumatoid arthritis was also tested, but the effect was not statistically significant (Linos *et al.* 1991). The study, although of great interest, has the drawback that the population studied consisted of a very large proportion of high consumers of olive oil. However, there is further evidence that olive oil may have beneficial effects relating to rheumatoid arthritis. In a study by Kremer *et al.* (1990) examining the effects of fish oil supplementation on the severity and progression of rheumatoid arthritis, olive oil was used as a placebo treatment, but clinical evaluations and immunological tests showed it to have effects which were similar to those of fish oil. A total of five of forty-five clinical measures were significantly changed from baseline in the olive oil group, eight of forty-five in a low-dose fish oil group and twenty-one of forty-five in a high-dose fish oil group (Kremer *et al.* 1990). Production of interleukin-1 by macrophages was decreased in the olive oil group, although not to the same extent as in either of the fish oil groups (Kremer *et al.* 1990). The authors concluded that 'dietary supplementation with olive oil is also associated with certain changes in immune function, which require further investigation.'

Conclusion

Animal studies, depending on the protocol, species and type of measurement, generally support the idea that olive oil is capable of modulating functions of cells of the immune system. The effects appear to be similar to, albeit weaker

Table 5. Effect of dietary lipids on popliteal lymph node (PLN) weight following the graft *v.* host and host *v.* graft response in the rat (Data taken from Sanderson *et al.* 1995*b*)

	Graft *v.* host PLN wt (mg)		Host *v.* graft PLN wt (mg)	
Diet	Mean	SEM	Mean	SEM
LF	$102{\cdot}7^{cf}$	8·2	$27{\cdot}5^{f}$	1·5
HCO	$101{\cdot}8^{f}$	14·9	$28{\cdot}6^{f}$	2·0
OO	$77{\cdot}3^{ae}$	7·5	$31{\cdot}3^{f}$	2·4
SO	$92{\cdot}3^{f}$	6·3	$30{\cdot}8^{f}$	2·0
EPO	$107{\cdot}4^{cf}$	6·3	$32{\cdot}5^{f}$	1·8
MO	$67{\cdot}8^{abde}$	5·7	$22{\cdot}0^{abcde}$	1·8

LF, low-fat; HCO, hydrogenated coconut oil; OO, olive oil; SO, safflower oil; EPO, evening primrose (*Oenothera biennis*) oil; MO, menhaden oil.
^{a,b,c,d,e,f} Mean values were significantly different (one-way ANOVA; $P < 0{\cdot}05$) for the following comparisons: ^{a} *v.* LF, ^{b} *v.* HCO, ^{c} *v.* OO, ^{d} *v.* SO, ^{e} *v.* EPO, ^{f} *v.* MO.

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than, those seen following feeding of diets containing fish oils. There is some evidence that the effects of olive oil on immune function in animal studies are due to oleic acid rather than to trace elements or antioxidants. Importantly, several studies have demonstrated effects of oleic acid-containing diets on *in vivo* immune responses.

In contrast, consumption of a MUFA-rich diet by human subjects does not appear to bring about a general suppression of immune cell functions. The effects of this type of diet in human subjects are limited to decreasing the expression of adhesion molecules on PBMNC (Yaqoob *et al.* 1998) and decreasing LDL-induced adhesion of monocytes to endothelial cells (Mata *et al.* 1996), although there are trends towards decreases in NK cell activity and proliferation (Yaqoob *et al.* 1998). The lack of a clear effect of MUFA in human subjects may be attributable to the higher level of monounsaturated fat used in the animal studies, as discussed previously; however, it is ultimately of importance to examine the effects of intakes which are in no way extreme. The intakes employed in the two human studies discussed closely correspond to current Mediterranean intakes and can readily be achieved through consumption of meals which use olive oil as the primary cooking fat.

Since the human studies concentrated on changes in macronutrient intake, the possibility that levels of trace elements or antioxidants varied between the diets and/or subjects cannot be excluded. Thus, the suggestion that the effects observed in these studies are due to specific modulation of dietary oleic acid is favourable (given the changes in fatty acid composition in both), but not conclusive. Similarly, it is extremely difficult to determine conclusively whether the effects observed are indeed due to an increased level of MUFA or to a decreased level of SFA. The effects of MUFA on adhesion molecules are potentially important, since they appear to have a role in the pathology of a number of diseases involving the immune system. This area clearly deserves further exploration.

References

Berger A, German JB, Chiang BL, Ansari AA, Keen CL, Fletcher MP & Gershwin MR (1993) Influence of feeding unsaturated fats on growth and immune status of mice. *Journal of Nutrition* **123**, 225–233.

Besler HT & Grimble RF (1995) Comparison of the modulatory influence of maize and olive oils and butter on metabolic responses to endotoxin in rats. *Clinical Science* **88**, 59–66.

Chapman PT & Haskard DO (1995) Leukocyte adhesion molecules. *British Medical Bulletin* **51**, 296–311.

Cleland LG, French JK, Betts WH, Murphy GA & Elliot MJ (1988) Clinical and biochemical effects of dietary fish oil supplements in rheumatoid arthritis. *Journal of Rheumatology* **15**, 1471–1475.

Cronstein BN (1994) Adhesion molecules in the pathogenesis of rheumatoid arthritis. *Current Opinions in Rheumatology* **6**, 300–304.

De Caterina R, Cybulsky MI, Clinton SK, Gimbrone MA Jr & Libby P (1994) The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arteriosclerosis and Thrombosis* **14**, 1829–1836.

Dehmer GJ, Popma JJ, van den Berg EK, Eichhorn EJ, Prewitt JB, Campbell WB, Jennings L, Willerson JT & Schmitz JM (1988) Reduction in the rate of early restenosis after coronary angioplasty by a diet supplemented with *n*-3 fatty acids. *New England Journal of Medicine* **319**, 733–740.

Ferro-Luzzi A & Branca F (1995) The Mediterranean diet, Italian style: prototype of a healthy diet. *American Journal of Clinical Nutrition* **61**, Suppl., 1338S–1345S.

Gunstone FD, Harwood JL & Padley FB (1994) *The Lipid Handbook*, 2nd ed., pp. 79–82. London: Chapman & Hall.

Gurr MI (1983) The role of lipids in the regulation of the immune system. *Progress in Lipid Research* **22**, 257–287.

Herberman RB (1988) *Inflammation: Basic Principles and Clinical Correlates*, pp. 613–630 [JI Gallin, IM Goldstein and R Snyderman, editors]. New York: Raven Press.

Jeffery NM, Cortina M, Newsholme EA & Calder PC (1997) Effects of variations in the proportions of saturated, monounsaturated and polyunsaturated fatty acids in the rat diet on spleen lymphocyte functions. *British Journal of Nutrition* **77**, 805–823.

Jeffery NM, Yaqoob P, Newsholme EA & Calder PC (1996) The effects of olive oil upon rat serum lipid levels and lymphocyte functions appear to be due to oleic acid. *Annals of Nutrition and Metabolism* **40**, 71–80.

Keys A (1970) Coronary heart disease in seven countries. *Circulation* **41**, 1–211.

Kremer JM, Lawrence DA, Jubiz W, DiGiacomo R, Rynes R, Bartholomew LE & Sherman M (1990) Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis and Rheumatism* **33**, 810–820.

Languino LR, Duperray A, Joganic KJ, Fornaro M, Thornton GB & Altieri DC (1995) Regulation of leukocyte-endothelium interaction and leukocyte transendothelial migration by intercellular adhesion molecule 1-fibrinogen recognition. *Proceedings of the National Academy of Sciences USA* **92**, 1505–1509.

Linos A, Kaklamanis E, Kontomerkos A, Koumantaki Y, Gazi S, Vaiopoulos G, Tsokos GC & Kaklamanis PH (1991) The effect of olive oil and fish consumption on rheumatoid arthritis – a case control study. *Scandinavian Journal of Rheumatology* **20**, 419–426.

Mata P, Alonso R, Lopez-Farre A, Ordovas JM, Lahoz C, Garces C, Caramelo C, Codoceo R, Blazquez E & de Oya M (1996) Effect of dietary fat saturation on LDL oxidation and monocyte adhesion to human endothelial cells in vitro. *Arteriosclerosis Thrombosis and Vascular Biology* **16**, 1347–1355.

Mata P, Alvarez-Silva LA, Rubio MJ, Nuno J & De Oya M (1992) Effects of long-term monounsaturated vs polyunsaturated-enriched diets on lipoproteins in healthy men and women. *American Journal of Clinical Nutrition* **55**, 846–850.

Mensink RP & Katan MB (1987) Effect of monounsaturated fatty acids versus complex carbohydrates on high-density lipoproteins in healthy men and women. *Lancet* **i**, 122–124.

Milner MR (1989) Fish oil for preventing coronary restenosis. *Lancet* **i**, 693.

Mulrooney HM & Grimble RF (1993) Influence of butter and of corn, coconut and fish oils on the effects of recombinant human tumour necrosis factor-α in rats. *Clinical Science* **84**, 105–112.

Munro JM (1993) Endothelial-leukocyte adhesive interactions in inflammatory diseases. *European Heart Journal* **14**, Suppl. K, 72–77.

Nestle M (1995) Mediterranean diets: historical and research overview. *American Journal of Clinical Nutrition* **61**, Suppl., 1313S–1320S.

Poston RN, Haskard DO, Coucher JR, Gall NP & Johnson-Tidy RR (1992) Expression of intercellular adhesion molecule-1 in atherosclerotic plaques. *American Journal of Pathology* **140**, 665–673.

Sanderson P, Yaqoob P & Calder PC (1995*a*) Effects of dietary lipid manipulation upon rat spleen lymphocyte functions and the expression of lymphocyte surface molecules. *Journal of Nutritional and Environmental Medicine* **5**, 119–132.

Sanderson P, Yaqoob P & Calder PC (1995*b*) Effects of dietary lipid manipulation upon graft vs host and host vs graft responses in the rat. *Cellular Immunology* **164**, 240–247.

Virella G, Fourspring K, Hyman B, Haskill-Stroud R, Long L, Virella I, La Via M, Gross AJ & Lopes-Virella M (1991) Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentaenoic acid on human lymphocytes. *Clinical Immunology and Immunopathology* **61**, 161–176.

Yaqoob P & Calder PC (1993) The effects of fatty acids on lymphocyte functions. *International Journal of Biochemistry* **25**, 1705–1714.

Yaqoob P, Knapper JA, Webb DH, Williams CM, Newsholme EA & Calder PC (1998) The effect of olive oil consumption on immune functions in middle-aged men. *American Journal of Clinical Nutrition* **67**, 129–135.

Yaqoob P, Newsholme EA & Calder PC (1994*a*) The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology* **82**, 603–610.

Yaqoob P, Newsholme EA & Calder PC (1994*b*) Inhibition of natural killer cell activity by dietary lipids. *Immunology Letters* **41**, 241–247.

Yaqoob P, Newsholme EA & Calder PC (1995*a*) The effect of fatty acids on leukocytes subsets and proliferation in whole blood. *Nutrition Research* **15**, 279–287.

Yaqoob P, Newsholme EA & Calder PC (1995*b*) Influence of cell culture conditions on diet-induced changes in lymphocyte fatty acid composition. *Biochimica et Biophysica Acta* **1255**, 333–340.