Monounsaturated fats and immune function

Parveen Yaqoob*

Institute of Human Nutrition, Biomedical Sciences Building, University of Southampton, Bassett Crescent East, Southampton SO16 7PX, UK

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 (Kremers 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 (mendaden) 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; MUF A, monounsaturated fatty acids; NK, natural killer; PBMC, peripheral-blood mononuclear cells; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; TNFα, tumour necrosis factor-α; VCAM-1, vascular-cell adhesion molecule-1.

Corresponding author: Dr Parveen Yaqoob, fax 44 (0) 118 931 0080, email P.Yaqoob@afnovell.reading.ac.uk

*Present address: Department of Food Science & Technology, PO Box 226, Whiteknights, Reading RG6 2AP, UK.
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. 1994a; 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. 1995a; 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. 1995b). 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. 1995b) and negates any effects of dietary lipid manipulation on cell function (Yaqoob et al. 1994a; 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. 1994a; Fig. 1) or of whole-blood cultures (Yaqoob et al. 1995a; 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

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**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 [3H]thymidine and expressed as stimulation index (incorporation of [3H]thymidine by stimulated cells/ incorporation of [3H]thymidine by unstimulated cells). 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 < 0.05. (Data taken from Yaqoob et al. 1994a.)

**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 μg-glutamine and antibiotics) in response to Con A was measured by incorporation of [3H]thymidine and expressed as stimulation index (incorporation of [3H]thymidine by stimulated cells/ incorporation of [3H]thymidine by unstimulated cells). 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 < 0.05. (Data taken from Yaqoob et al. 1995a.)
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; 

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

where \(a_0\) is 75.8 and \(b_0\) is -18.56 (\(P < 0.05\) 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/m²) 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 (incorporation of \(\text{[^{3}H]thymidine}\) by stimulated cells) / (incorporation of \(\text{[^{3}H]thymidine}\) by unstimulated cells). 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. safflower oil (SO), § v. HOSO. (Data taken from Jeffery et al. 1996.)](https://www.cambridge.org/core/journals/lipids-and-the-immune-system/article/the-effect-of-feeding-a-high-oleic-sunflower-oil-hoso-diet-on-proliferation-of-rat-spleen-lymphocytes/677D5A3F74D065F6822C2E5E6A50D588)

![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 (incorporation of \(\text{[^{3}H]thymidine}\) by stimulated cells) / (incorporation of \(\text{[^{3}H]thymidine}\) by unstimulated cells). Points are means with their standard errors represented by vertical bars. (Data taken from Jeffery et al. 1997.)](https://www.cambridge.org/core/journals/lipids-and-the-immune-system/article/relationship-between-the-oleic-acid-linoleic-acid-oa-la-in-the-diet-and-spleen-lymphocyte-proliferation-in-rats/5E39C34F5FBCB2CB1DB02FBF903C5B29)
mononuclear cells (PBMC; 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. 1994a, 1995a). 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 fish oil/kg (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 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. 1994b; 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
Table 2. The effect of dietary lipid manipulation on natural killer activity in freshly-prepared rat spleen lymphocytes
(Data taken from Yaqoob et al. 1994b)

<table>
<thead>
<tr>
<th>Diet</th>
<th>E : T...</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
<th>Mean</th>
<th>SEM</th>
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<tbody>
<tr>
<td>LF</td>
<td>100 : 1</td>
<td>46.5</td>
<td>2.0</td>
<td>29.7</td>
<td>2.8</td>
<td>17.7</td>
<td>0.6</td>
<td>7.4</td>
<td>0.6</td>
</tr>
<tr>
<td>HCO</td>
<td>50 : 1</td>
<td>40.4</td>
<td>2.4</td>
<td>22.9</td>
<td>1.5</td>
<td>11.5</td>
<td>0.9</td>
<td>4.5</td>
<td>0.8</td>
</tr>
<tr>
<td>OO</td>
<td>25 : 1</td>
<td>36.4</td>
<td>2.8</td>
<td>20.8</td>
<td>1.7</td>
<td>9.3</td>
<td>0.9</td>
<td>3.4</td>
<td>0.4</td>
</tr>
<tr>
<td>SO</td>
<td>12.5 : 1</td>
<td>42.1</td>
<td>1.5</td>
<td>24.2</td>
<td>1.3</td>
<td>11.2</td>
<td>0.7</td>
<td>3.9</td>
<td>0.7</td>
</tr>
<tr>
<td>EPO</td>
<td></td>
<td>40.7</td>
<td>0.7</td>
<td>19.1</td>
<td>2.6</td>
<td>8.6</td>
<td>0.2</td>
<td>3.2</td>
<td>0.4</td>
</tr>
<tr>
<td>MO</td>
<td></td>
<td>29.4</td>
<td>2.1</td>
<td>13.9</td>
<td>0.7</td>
<td>5.0</td>
<td>0.3</td>
<td>3.1</td>
<td>0.8</td>
</tr>
</tbody>
</table>

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.

Mean values were significantly different ($P＜0.05$) for the following comparisons: * v. low-fat (LF), † v. olive oil (OO), § v. sunflower oil (SO), ‡ v. high-oleic sunflower oil (HOSO). (Data taken from Jeffery et al. 1996.)

![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.)](https://doi.org/10.1079/PNS19980075)

![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.)](https://doi.org/10.1079/PNS19980075)

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. 1994b) 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...
Table 3. Effect of monounsaturated fatty acid (MUFA) consumption on natural killer cell activity of peripheral-blood mononuclear cells (PBMC) 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 (PBMC). 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)

<table>
<thead>
<tr>
<th>Period on MUFA</th>
<th>Control (19)</th>
<th>MUFA (21)</th>
<th>Control (25)</th>
<th>MUFA (24)</th>
<th>Control (15)</th>
<th>MUFA (19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E : T</td>
<td>Mean SEM</td>
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<td>Mean SEM</td>
<td>Mean SEM</td>
<td>Mean SEM</td>
<td>Mean SEM</td>
</tr>
<tr>
<td>100 : 1</td>
<td>60.9 ± 4.2</td>
<td>58.8 ± 4.6</td>
<td>63.7 ± 3.2</td>
<td>58.7 ± 4.0</td>
<td>63.0 ± 9.3</td>
<td>52.4 ± 4.5</td>
</tr>
<tr>
<td>50 : 1</td>
<td>41.5 ± 3.6</td>
<td>40.7 ± 3.3</td>
<td>43.3 ± 3.1</td>
<td>36.9 ± 3.9</td>
<td>43.2 ± 4.9</td>
<td>34.2 ± 3.4</td>
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<tr>
<td>25 : 1</td>
<td>28.6 ± 3.4</td>
<td>25.1 ± 2.8</td>
<td>28.0 ± 2.4</td>
<td>24.2 ± 2.8</td>
<td>25.4 ± 2.9</td>
<td>23.5 ± 3.3</td>
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<tr>
<td>12.5 : 1</td>
<td>18.8 ± 2.5</td>
<td>15.2 ± 2.0</td>
<td>18.5 ± 1.7</td>
<td>14.3 ± 1.8</td>
<td>16.0 ± 2.2</td>
<td>13.4 ± 2.4</td>
</tr>
</tbody>
</table>

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 PBMC (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 transendothelial migration of leucocytes into synovial tissue and fluid in rheumatoid arthritis and in leucocyte-endothelium interactions which lead to the formation of atherosclerotic plaques (Monro, 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 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 mononuclear 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. 1995a). 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
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)

<table>
<thead>
<tr>
<th>Period on MUFA...</th>
<th>Baseline (18–20)</th>
<th>MUFA (19–20)</th>
<th>1 month</th>
<th>MUFA (20–23)</th>
<th>2 months</th>
<th>Statistical significance of difference between groups (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface marker</td>
<td>Control</td>
<td>MUFA</td>
<td>Control</td>
<td>MUFA</td>
<td>Control</td>
<td></td>
</tr>
</tbody>
</table>
| ICAM-1            | Mean             | SEM          | Mean    | SEM          | Mean     | SEM          | Mean        | SEM          | Mean        | SEM          | Mean        | SEM          | Significant effect of diet $=$ 0.035, and time $P = 0.049$, with a significant interaction $P = 0.046$.
| Mac-1             | Mean             | SEM          | Mean    | SEM          | Mean     | SEM          | Mean        | SEM          | Mean        | SEM          | Mean        | SEM          | No significant effect of time or diet or interaction between time and diet as analysed by two-way repeat-measures ANOVA.

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): $**P < 0.001$. 
Mean value was significantly different from baseline value (Student’s t-test): $*$P < 0.05. 
$*$ 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.

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 PBMCN 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. (1995b) 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. 1995b). 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. 1995b). 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 interindividual 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
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 PBMCN (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


