

## Effect of olive oil on immune function in middle-aged men<sup>1-3</sup>

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**ABSTRACT** Consumption of diets rich in monounsaturated fatty acids (MUFAs) has been linked with a low prevalence of atherosclerosis and there has been great interest in the effects of MUFAs on lipoprotein metabolism. Less attention has been paid to the effects of MUFAs on the immune system, yet cells of the immune system are an inherent part of the inflammatory events involved in atherosclerosis and several animal studies showed that olive oil has some potent immunomodulatory actions. We therefore considered it important to investigate the effects of chronic consumption of MUFAs on several immune cell functions in healthy humans. Healthy middle-aged males entered a double-blind, randomized, controlled trial in which they consumed either a MUFA diet or a control diet for 2 mo. There was a significant decrease in the expression of intercellular adhesion molecule 1 by peripheral blood mononuclear cells from subjects consuming the MUFA diet. Consumption of the MUFA diet did not affect natural killer cell activity or proliferation of mitogen-stimulated leukocytes. The effects of a MUFA-rich diet on adhesion molecule expression may have implications for the influence of dietary fat on inflammatory diseases, including atherosclerosis. *Am J Clin Nutr* 1998;67:129–35.

**KEY WORDS** Monounsaturated fatty acids, lipids, immunity, olive oil, adhesion molecules, men, intercellular adhesion molecule 1, natural killer cell activity, Mediterranean diet, atherosclerosis

### INTRODUCTION

The Mediterranean diet has become a cultural model for dietary improvement. Since the 1950s evidence has been growing that Mediterranean countries display rates of chronic diseases that are among the lowest in the world and life expectancies that are among the highest (1). 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 use of olive oil is strongly correlated with a low intake of saturated fat (2). As the Seven Countries Study (2) clearly showed, it is the type of fat rather than the amount of fat consumed in the diet that is most closely related to the incidence of coronary artery disease; subsequent studies showed that the replacement of saturated fatty acids (SFAs) with either monounsaturated fatty acids (MUFAs) or polyunsaturated fatty acids (PUFAs) may be beneficial (3, 4).

Because the consumption of diets rich in MUFAs has been linked with a low prevalence of atherosclerosis, there has been great interest in the effects of MUFAs on lipoprotein metabolism (4). Less attention has been paid to the effects of MUFAs on the

immune system, yet cells of the immune system are an inherent part of the inflammatory events involved in the development and progression of atherosclerosis. Previous work in laboratory rodents showed that feeding a diet rich in olive oil suppresses natural killer cell activity (5), mitogen-stimulated proliferation (6, 7), and the expression of receptors for interleukin 2 and transferrin (6) in spleen lymphocyte preparations. Comparison of the effects of feeding olive oil, safflower oil, and a high-oleic acid sunflower oil on these immune cell functions suggested that the effects were due to oleic acid rather than to a nonlipid component of olive oil (8).

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, because MUFAs were typically regarded as being neutral fatty acids (9, 10). However, several clinical trials have reported effects of the olive oil treatment. For example, Oosthuizen et al (11) reported a lowering of plasma fibrinogen in women with high baseline fibrinogen concentrations by both fish oil and olive oil in a double-blind crossover study. Another study reported no significant difference between fish-oil supplements and an olive oil placebo in preventing restenosis after coronary angioplasty (12); a subsequent letter to *The Lancet* suggested that “future studies of oil supplements should not consider olive oil as a placebo until there are more data evaluating the role of MUFA in retarding atherosclerosis” (13).

A smaller number of studies suggested that there may be beneficial effects of olive oil consumption on rheumatoid arthritis (14), 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 (15) showed that frequent consumption of olive oil decreases the relative risk for developing rheumatoid arthritis (although unfortunately the study was limited to a single population in Greece). It is proposed that the suppressive effect of olive oil on the development of rheumatoid arthritis may be

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exerted via an effect on the immune system. Together with the evidence of immunosuppressive effects of olive oil from animal studies, we considered there to be a strong case for investigating the effects of chronic consumption of MUFAs on several immune cell functions in healthy humans.

## SUBJECTS AND METHODS

### Materials

Phosphate-buffered saline (PBS) tablets were obtained from Unipath Ltd (Basingstoke, United Kingdom). Histopaque, bovine serum albumin (fatty acid free), HEPES-buffered RPMI medium, glutamine, antibiotics (penicillin and streptomycin), and concanavalin A were obtained from Sigma Chemical Co (Poole, United Kingdom). Sodium azide was from BDH Chemicals Ltd (Poole, United Kingdom), [<sup>3</sup>H]thymidine was from Amersham International (Bucks, United Kingdom), cytotoxicity kits were from Proteins International Inc (Rochester, MI), and monoclonal antibodies and RAMFITC were from Serotec (Kidlington, United Kingdom). K562 cells were a gift from the Sir William Dunn School of Pathology, University of Oxford, Oxford, United Kingdom.

### Subjects and study design

Middle-aged men [mean age: 48 y; range: 41–56 y; body mass index (BMI; in kg/m<sup>2</sup>) range: 21.9–30.7] who were resident in the United Kingdom were randomly assigned to collect either a MUFA diet or a control diet. Thirty subjects were assigned to each group. Although no subjects dropped out or missed a sampling date, it was not possible for the Oxford group to collect blood samples from every subject at every time point; the results described in this paper therefore correspond to  $n = 19$ –29 for the MUFA group and  $n = 18$ –29 for the control group. Subjects were screened and excluded if fasting (12 h) plasma glucose was >6.2 mmol/L, triacylglycerol was >2.5 mmol/L, or total cholesterol was >6.5 mmol/L. All subjects were nonsmokers, were not vegetarian, and were not taking any medication or dietary supplements. All procedures involving human subjects were approved by the appropriate ethical committee.

Subjects consumed either a control diet (designed to reproduce the current fatty acid composition of the UK diet) or a diet containing foods enriched with highly refined olive oil for 8 wk. Foods provided for subjects included the main meal of the day (as a frozen meal), cooking oils and spreads, biscuits, and puddings. Fats were blended to provide the required fatty acid compositions by Unilever Ltd (Vlaardingen, Netherlands) and cooking fats and spreads were manufactured and produced by Van den Bergh Foods Ltd (Croydon, United Kingdom). Ready meals, frozen breads, and puddings were produced by using the oils and spreads described above by J Sainsbury plc (London) and biscuits by Diet and Health Biscuits Ltd (Sheffield, United Kingdom). All products and blood samples were coded at the University of Surrey, Surrey, United Kingdom. The meals, fats, and spreads were analyzed for their fatty acid composition and the data added to the FOODBASE database (Institute of Brain Chemistry, University of North London, London).

Compliance was assessed with 5-d food diaries, questionnaires, and analysis of plasma phospholipid fatty acid composition. Actual dietary intakes were calculated with FOODBASE, a dietary analysis package produced by the Institute of Brain Chemistry.

The study was organized and conducted at the University of Surrey. Blood samples were collected after a 12-h (overnight) fast at baseline and after 1 and 2 mo of consuming the diets and were delivered to the University of Oxford by courier, normally within 2 h.

### Analysis of fatty acid composition

The fatty acid composition of plasma total phospholipids and of peripheral blood mononuclear cell (PBMC) total lipids (in samples that contained sufficient material once all other experiments had been performed) was analyzed as described elsewhere (16).

### Preparation of PBMCs

Fasting blood samples (30 mL) were collected into heparin-coated tubes and 25 mL was diluted 1:1 with PBS. The diluted blood was layered onto histopaque (density: 1.077 g/L; ratio of diluted blood to histopaque, 4:3) and centrifuged for 15 min at room temperature at  $800 \times g$ . The cells were washed once with PBS, resuspended in 2.5 mL PBS, and layered onto 5 mL histopaque. They were centrifuged once more to achieve a lower degree of erythrocyte contamination, washed with PBS, and finally resuspended in the appropriate medium.

### Analysis of surface molecule expression

Approximately  $1 \times 10^6$  PBMCs, resuspended in PBS supplemented with 0.1% (wt:vol) bovine serum albumin and 10 mmol sodium azide/L, were analyzed for the expression of CD7, CD3, CD2, CD21, CD4, CD8, CD54, CD11a, CD11b, CD64, and CD16 by fluorescence-activated cell sorter analysis as described elsewhere (6). Fluorescence data were collected on  $5 \times 10^3$  viable cells and are expressed as a percentage of marker-positive cells.

### Measurement of natural killer cell activity

The activity of natural killer cells against the K562 cell line in preparations of PBMCs was determined by measuring the release of cellular lactate dehydrogenase (LDH) on target cell lysis by using a kit. PBMCs were prepared as described above and resuspended in HEPES-buffered RPMI supplemented with 2 mmol glutamine/L and antibiotics. K562 cells were washed three times and resuspended in HEPES-buffered RPMI supplemented with 1% bovine serum albumin and antibiotics. Effector cells (PBMCs) and target cells (K562 cells) were added to each well of a 96-well microtiter plate at effector-to-target cell ratios of 100:1, 50:1, 25:1, and 12.5:1 (final volume: 150  $\mu$ L). Maximal target cell lysis was assessed by incubating K562 cells with lysing reagent, provided in the cytotoxicity kit. Spontaneous release of LDH by PBMCs was assessed by incubating the cells in the absence of target cells and spontaneous release of LDH by K562 cells was assessed by incubating these cells in the absence of effector cells. Plates were centrifuged for 3 min at 5–10 °C at  $\approx 100 \times g$  to bring effector and target cells into contact and were then incubated at 37 °C in an air-CO<sub>2</sub> (19:1) atmosphere for 4 h. After the plates were incubated, 50  $\mu$ L ice-cold K562 medium was added to each well and the plates centrifuged for 5 min at  $400 \times g$  at 5–10 °C. A sample (100  $\mu$ L) of medium was removed from each well and transferred to the wells of a flat-bottomed 96-well plate. The activity of LDH in the medium was assayed according to the instructions provided by the manufacturers of the kit. Percentage cytolysis was calculated as

$$\text{Cytolysis (\%)} = \frac{\text{experimental LDH} - \text{spontaneous (PBMC) LDH release}}{\text{maximal (K562) LDH release} - \text{spontaneous (K562) LDH release}} \times 100 \quad (1)$$

**TABLE 1**  
Calculated dietary intakes<sup>1</sup>

	Control diet	MUFA diet
Energy (kJ)	11 975 ± 142	10 874 ± 50
Fat (% of energy)	36.1 ± 0.1	37.7 ± 0.0
Protein (% of energy)	13.9 ± 0.4	14.7 ± 0.2
Carbohydrate (% of energy)	49.2 ± 0.3	46.2 ± 0.1
SFAs (% of energy)	13.3 ± 0.1	10.0 ± 0.1 <sup>2</sup>
MUFAs (% of energy)	11.3 ± 0.1	18.4 ± 0.1 <sup>2</sup>
PUFAs (% of energy)	6.9 ± 0.2	7.1 ± 0.2
Ratio of 16:0 to 18:0	2.2 ± 0.0	2.2 ± 0.0
Ratio of 18:2 to 18:3	17.0 ± 1.1	18.2 ± 2.0
Ratio of 18:0 to 18:1	0.4 ± 0.0	0.2 ± 0.0 <sup>2</sup>

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ ;  $n = 30$  per group. MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid.

<sup>2</sup> Significantly different from control diet,  $P < 0.01$  (ANOVA).

### Measurement of cell proliferation in whole blood

Blood (0.45 mL) was diluted 1:10 with HEPES-buffered RPMI supplemented with 2 mmol glutamine/L and antibiotics and proliferation measured as described previously (7). Briefly, the diluted blood (180  $\mu\text{L}$ ) was cultured in 96-well microtiter plates with 20  $\mu\text{L}$  of the T cell mitogen concanavalin A at a range of concentrations between 10 and 100 mg/L at 37°C in an atmosphere of air and CO<sub>2</sub> (19:1). Incorporation of [<sup>3</sup>H]thymidine was measured over the final 18 h of a 66-h culture period. Thymidine incorporation data are expressed as stimulation index (SI) values (incorporation in the presence of mitogen divided by incorporation in the absence of mitogen).

### Measurement of proliferation of PBMCs

PBMCs ( $2 \times 10^9/\text{L}$ ) were cultured in HEPES-buffered RPMI supplemented with 2 mmol glutamine/L, 2.5% (by vol) autologous plasma, antibiotics, and, for stimulated cells, 15 mg concanavalin A/L. Proliferation was assessed as described above.

### Statistical analysis

Results are expressed as means  $\pm$  SEMs for the number of observations indicated. The statistical significance of diet and

time and their interaction was determined with use of two-way repeated-measures analysis of variance. When significant to the level of  $P < 0.05$ , data were analyzed further by the unpaired Student's  $t$  test, with use of Dunnett's tables for comparisons.

## RESULTS

### Dietary intake of subjects

Dietary intakes of subjects in both experimental groups were close to those desired. Control subjects consumed a diet with a fatty acid composition that closely resembled that of the current UK intake (Table 1). Subjects consuming the MUFA diet consumed significantly less saturated fat (as a percentage of energy) than did those consuming the control diet and significantly more MUFAs; MUFAs contributed 18.4% of energy in this group compared with 11.3% in the control group (Table 1). Intakes of fatty acids other than SFAs and MUFAs did not differ between diets and the ratios of palmitic acid (16:0) to stearic acid (18:0) and of linoleic acid (18:2) to  $\alpha$ -linolenic acid (18:3) were similar (Table 1).

### Fatty acid composition of plasma phospholipids and PBMCs

For subjects consuming the MUFA diet there was a gradual and significant increase in the proportion of oleic acid (18:1) in plasma phospholipids; by 1 mo on this diet the proportion of oleic acid was significantly greater than at baseline and than in the control group (Table 2). Some of this increase may have been accounted for by a small decrease in the proportion of palmitic acid in the MUFA group at 2 mo compared both with the control group and with baseline values (Table 2).

PBMCs from subjects consuming the control diet showed no changes in the fatty acid composition of total lipids throughout the experimental period (Table 3). After 2 mo (but not 1 mo) of consuming the MUFA-enriched diet, subjects in the MUFA group had a significantly higher proportion of oleic acid in their PBMC total lipids than did those in the control group (Table 3).

**TABLE 2**  
Effect of monounsaturated fatty acid (MUFA) consumption on plasma phospholipid fatty acid composition<sup>1</sup>

	Baseline		1 mo		2 mo		ANOVA <sup>2</sup>
	Control diet ( $n = 19$ )	MUFA diet ( $n = 23$ )	Control diet ( $n = 22$ )	MUFA diet ( $n = 25$ )	Control diet ( $n = 19$ )	MUFA diet ( $n = 22$ )	
	% by wt						
16:0	36.3 ± 0.9	34.8 ± 0.8	35.9 ± 0.6	35.1 ± 0.7	35.0 ± 1.0	32.4 ± 0.7 <sup>3,4</sup>	a
16:1	1.1 ± 0.2	1.0 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	NS
18:0	17.1 ± 0.4	17.0 ± 0.5	18.1 ± 0.6	17.8 ± 0.4	17.7 ± 0.6	17.1 ± 0.4	NS
18:1	11.8 ± 0.5	12.0 ± 0.3	11.7 ± 0.3	13.5 ± 0.4 <sup>3,6</sup>	11.7 ± 0.4	14.1 ± 0.34 <sup>5,6</sup>	b
18:2	23.0 ± 0.6	22.9 ± 0.6	24.1 ± 0.8	23.0 ± 0.5	23.9 ± 0.7	23.3 ± 0.6	NS
20:4	5.4 ± 0.3	5.7 ± 0.4	4.9 ± 0.4	5.3 ± 0.4	5.7 ± 0.4	5.7 ± 0.5	NS
22:6	0.8 ± 0.2	1.1 ± 0.2	0.9 ± 0.2	0.6 ± 0.1	1.0 ± 0.1	0.8 ± 0.2	NS

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ .

<sup>2</sup> Two-way, repeated-measures ANOVA: a, significant effect of time ( $P = 0.024$ ) and diet ( $P = 0.006$ ) with no interaction ( $P = 0.600$ ); b, significant effect of time ( $P = 0.019$ ) and diet ( $P = 0.001$ ) with a significant interaction ( $P = 0.015$ ).

<sup>3,5</sup> Significantly different from control group (Dunnett's test): <sup>3</sup>  $P < 0.05$ , <sup>5</sup>  $P < 0.01$ .

<sup>4,6</sup> Significantly different from baseline (Dunnett's test): <sup>4</sup>  $P < 0.05$ , <sup>6</sup>  $P < 0.01$ .

**TABLE 3**Effect of monounsaturated fatty acid (MUFA) consumption on the fatty acid composition of peripheral blood mononuclear cell total lipids<sup>1</sup>

	Baseline		1 mo		2 mo		ANOVA <sup>2</sup>
	Control diet (n = 10)	MUFA diet (n = 10)	Control diet (n = 8)	MUFA diet (n = 12)	Control diet (n = 10)	MUFA diet (n = 14)	
	% by wt						
14:0	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	NS
16:0	24.6 ± 1.2	23.9 ± 1.1	24.6 ± 1.5	25.1 ± 1.4	25.9 ± 1.3	25.1 ± 0.8	NS
16:1	0.2 ± 0.1	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	NS
18:0	29.8 ± 1.5	29.7 ± 1.4	33.4 ± 1.3	32.2 ± 1.2	33.8 ± 1.6	32.0 ± 1.2	NS
18:1	21.3 ± 0.8	22.9 ± 1.0	20.4 ± 0.7	22.8 ± 1.0	20.3 ± 1.0	24.5 ± 0.8 <sup>3</sup>	a
18:2	6.3 ± 0.7	6.6 ± 0.4	7.9 ± 0.8	7.0 ± 0.8	6.8 ± 0.5	7.3 ± 0.7	NS
20:4	12.7 ± 2.3	9.0 ± 2.1	10.3 ± 2.1	8.7 ± 1.6	9.8 ± 1.9	7.6 ± 1.3	NS

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ .<sup>2</sup> Two-way, repeated-measures ANOVA: a, significant effect of diet ( $P = 0.001$ ) but not time ( $P = 0.774$ ) with no interaction ( $P = 0.091$ ).<sup>3</sup> Significantly different from control group,  $P < 0.01$  (Dunnnett's test).**Effect of MUFAs on expression of surface molecules on PBMCs**

There was no effect of either the control or the MUFA diet on the expression of CD7, CD3, CD2, CD21, CD4, CD8, or CD64 by PBMCs (data not shown). After 2 mo the MUFA diet resulted in a significant decrease in the expression of the leukocyte adhesion molecule ICAM-1 (CD54) compared with baseline values (Table 4). After 2 mo of consumption of the MUFA diet, the proportion of cells expressing ICAM-1 was also significantly lower than in the control group at the same time point; the expression of ICAM-1 did not change during consumption of the control diet. The MUFA diet also tended to decrease the expression of a monocyte- and macrophage-associated adhesion molecule, Mac-1 (CD11b), by  $\approx 25\%$  compared with baseline values. However, this decrease was not statistically significant, either compared with baseline or with the control group. The expression of the natural killer cell marker CD16 was significantly decreased at 2 mo in both the control group and the MUFA group so that there was no difference between the two dietary groups at any time point.

**Effect of MUFAs on natural killer cell activity**

Natural killer cell activity was unaffected by consumption of the control diet (Table 5). Consumption of the MUFA diet tended to produce a small decrease in natural killer cell activity at 2 mo but not 1 mo. However, this was not significant

when compared either with baseline or with the control group, largely because of the small sample size for the control group at 2 mo.

**Effect of MUFAs on proliferation of leukocytes**

The use of a whole-blood culture system avoids the removal of erythrocytes, platelets, granulocytes, and monocytes, some or all of which may be lost during the isolation of PBMCs. It also avoids the removal of noncellular components normally in contact with the cells in the circulation, including growth factors and hormones. Importantly, the technique has the advantage that the ratios between different cell types and between cells and noncellular components are the same in vitro as in vivo. The proliferation of cells in whole-blood cultures, stimulated by the T cell mitogen concanavalin A, was unaffected by consumption of either the control diet (Figure 1A) or the MUFA diet (Figure 1B). The apparent decrease in the proliferative response to concanavalin A in the MUFA group at the higher mitogen concentrations at 2 mo compared with baseline values was not significant (Figure 1B). There was also no significant difference in the proliferation of whole blood in the MUFA group compared with the control group at 2 mo (Figure 2). The proliferation of PBMCs, cultured in the presence of autologous plasma and 15 mg concanavalin A/L, was unaffected by either the control diet or the MUFA diet (Figure 3).

**TABLE 4**Effect of monounsaturated fatty acid (MUFA) consumption on expression of surface molecules on peripheral blood mononuclear cells<sup>1</sup>

	Baseline		1 mo		2 mo		ANOVA <sup>2</sup>
	Control diet (n = 18–20)	MUFA diet (n = 19–20)	Control diet (n = 18–23)	MUFA diet (n = 20–23)	Control diet (n = 20–23)	MUFA diet (n = 19–21)	
	% marker-positive cells						
CD54	19.0 ± 1.3	20.8 ± 1.4	19.1 ± 1.2	16.4 ± 1.4	20.0 ± 1.5	15.9 ± 1.1 <sup>3,4</sup>	a
CD11b	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
CD16	18.6 ± 1.6	16.8 ± 1.5	16.6 ± 1.6	17.4 ± 1.5	11.1 ± 1.0 <sup>4</sup>	11.7 ± 0.9 <sup>4</sup>	b

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ .<sup>2</sup> Two-way, repeated-measures ANOVA: a, significant effect of diet ( $P = 0.035$ ) and time ( $P = 0.049$ ) with a significant interaction ( $P = 0.046$ ); b, significant effect of time ( $P = 0.001$ ) but not diet ( $P = 0.609$ ) with no interaction ( $P = 0.546$ ).<sup>3</sup> Significantly different from control group,  $P < 0.05$  (Dunnnett's test).<sup>4</sup> Significantly different from baseline,  $P < 0.05$  (Dunnnett's test).

TABLE 5

Effect of monounsaturated fatty acid (MUFA) consumption on natural killer cell activity of peripheral blood mononuclear cells<sup>1</sup>

Ratio of effector to target cells	Baseline		1 mo		2 mo	
	Control diet (n = 19)	MUFA diet (n = 21)	Control diet (n = 25)	MUFA diet (n = 24)	Control diet (n = 15)	MUFA diet (n = 19)
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

<sup>1</sup>  $\bar{x} \pm \text{SEM}$ . Two-way, repeated-measures ANOVA showed no significant effect of diet or time.

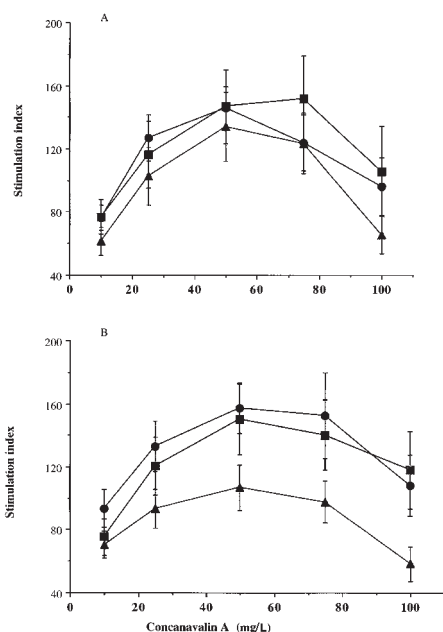
## DISCUSSION

This study showed that 2 mo of a MUFA-rich diet (containing MUFAs as 18.4 ± 0.1% of total energy) is sufficient to alter the fatty acid composition of plasma phospholipids and PBMC total lipids and reinforced the point that olive oil should not be considered as a placebo in clinical or cell biology studies. An important finding of the study was a decrease in the expression of ICAM-1 with the MUFA diet. ICAM-1 is a member of the immunoglobulin superfamily of adhesion molecules and is involved in leukocyte-leukocyte adhesion (17) as well as in adhesion of leukocytes to endothelial cells (18) and to fibrinogen, a plasma-adhesive protein (19). ICAM-1 is expressed on mononuclear cells that infiltrate inflamed synovium in patients suffering from rheumatoid arthritis (20); in some cases, such

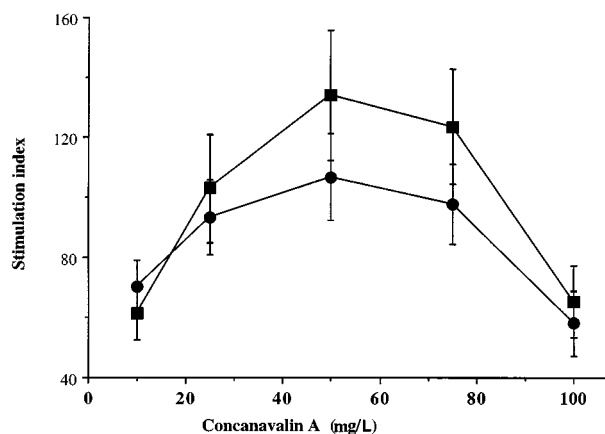
patients may also have high concentrations of serum-soluble ICAM-1 (20). The formation of plaques in atherosclerosis has many features in common with the inflammation seen in rheumatoid arthritis, such as adhesive interactions between endothelial cells and leukocytes and extravascular leukocyte accumulation. ICAM-1 is thought to play a pivotal role in the recruitment of (and therefore in the growth of) mononuclear cells to the atherosclerotic plaque (21). The fact that a MUFA diet appeared to decrease the expression of ICAM-1 on circulating PBMCs by up to 20% suggests the exciting possibility that the low prevalence of atherosclerosis, and perhaps of other inflammatory conditions, in Mediterranean populations may at least partly involve the effects of dietary MUFA on adhesion molecule expression.

The effects may not be limited to ICAM-1. In the present study, the effect of chronic MUFA consumption on the expression of Mac-1, a monocyte- and macrophage-associated adhesion molecule, was also investigated. Mac-1 is also thought to have a role in the pathophysiology of inflammation and has been detected on leukocytes in synovial tissue and fluid (22). We showed a modest decrease in the expression of Mac-1 with MUFA consumption. Because studies by De-Caterina and Libby (23) showed that oleic acid can reduce the expression of vascular cell adhesion molecule 1 by endothelial cells in vitro, this area of study clearly deserves further exploration.

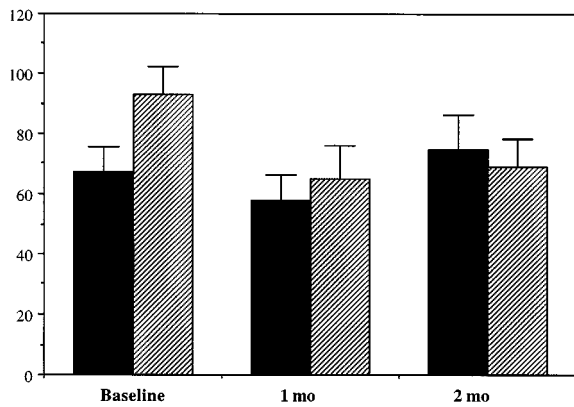
The present study showed that natural killer cell activity was not significantly affected by consumption of the MUFA diet. This observation contrasts with animal studies that showed a strong



**FIGURE 1.** Effect of monounsaturated fatty acid (MUFA) consumption on proliferation of leukocytes in whole blood at baseline (■), 1 mo (●), and 2 mo (▲) in the control group (A) and MUFA group (B). As described in the methods, blood was diluted and cultured with concanavalin A and incorporation of [<sup>3</sup>H]thymidine was measured over the final 18 h of a 66-h culture period. Thymidine incorporation is expressed as a stimulation index. Proliferation in unstimulated wells (as cpm) for the control group was 87.1 ± 9.3, 101.1 ± 11.1, and 93.0 ± 10.0 (at baseline, 1 mo, and 2 mo, respectively) and for the MUFA group was 86.3 ± 8.2, 108.3 ± 10.8, and 94.8 ± 8.0, respectively.  $\bar{x} \pm \text{SEM}$ ; n = 22–29 subjects per group. Significant effects of time and diet and their interactions were analyzed by two-way repeat-measures ANOVA.



**FIGURE 2.** Effect of the control (■) compared with the monounsaturated fatty acid (●) diet on the proliferation of leukocytes at 2 mo. Proliferation was assessed as described for Figure 1 and in the Methods.  $\bar{x} \pm \text{SEM}$ ; n = 24–29 subjects per group.




**FIGURE 3.** Effect of the control (■) compared with the monounsaturated fatty acid (MUFA; ▨) diet on proliferation of peripheral blood mononuclear cells. Proliferation in unstimulated wells (as cpm) for the control group was  $417 \pm 67$ ,  $591 \pm 105$ , and  $488 \pm 78$  (at baseline, 1 mo, and 2 mo, respectively) and for the MUFA group was  $238 \pm 34$ ,  $602 \pm 152$ , and  $469 \pm 74$ , respectively.  $\bar{x} \pm \text{SEM}$ ;  $n = 21\text{--}29$  subjects per group. Significant effects of time and diet and their interactions were analyzed by two-way repeated-measures ANOVA.

suppression of natural killer cell activity by a diet rich in olive oil (5). The difference may be attributable to the higher amount of monounsaturated fat used in the animal studies; these studies used 20% olive oil by weight (MUFAs therefore contributed  $\approx 30\%$  of total energy intake) fed to rats for 10 wk, whereas in the present study MUFAs contributed  $\approx 18\%$  of the total energy intake. Even at this intake, a small decrease in natural killer cell activity in the MUFA group at 2 mo was detected; however, this was not significant. Although it is possible that a higher MUFA intake may have resulted in suppression of immune cell functions, the purpose of this study was to examine the effects of intakes that are in no way extreme. The intakes described in the present study correspond closely with current Mediterranean intakes (24) and can readily be achieved through consumption of meals in which olive oil is used as the primary cooking fat.

Consumption of a MUFA-rich diet did not affect the proliferative response of cells, either in whole-blood cultures or in PBMCs, to the T cell mitogen concanavalin A. This observation also contrasts with results obtained in laboratory animals (6, 7). The lack of a clear effect of MUFAs on the proliferative response to concanavalin A in the present study may once again be due to the lower intake in the human study than in the animal studies. However, note that the small changes in natural killer cell activity and proliferation observed after 2 mo of consumption of the MUFA diet were accompanied by a significant increase in the proportion of oleic acid in PBMCs.

In conclusion, we showed that consumption of a MUFA-rich diet decreased the expression of some adhesion molecules by human PBMCs. However, the effects of this diet (containing an amount of MUFAs typical of that consumed in a Mediterranean diet) do not appear to involve a general suppression of immune cell functions. Because the present study concentrated on changes in macronutrient intake, we have not investigated the possibility that amounts of trace elements or antioxidants varied between the diets or subjects. Therefore, the suggestion that the effects observed in this study are due to specific modulation of dietary oleic acid is favorable (given the changes in fatty acid composi-

tion), but not conclusive. Similarly, it is difficult to determine conclusively whether the effects observed were indeed due to an increased amount of MUFAs or to a decreased amount of SFAs. The effects of MUFAs on adhesion molecules are potentially important because MUFA-rich diets appear to have a role in the pathology of several diseases involving the immune system. This area clearly deserves further exploration. 

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