Olive Oil and Modulation of Cell Signaling in Disease Prevention

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ABSTRACT: Epidemiological studies show that populations consuming a predominantly plant-based Mediterranean-style diet exhibit lower incidences of chronic diseases than those eating a northern European or North American diet. This observation has been attributed to the greater consumption of fruits and vegetables and the lower consumption of animal products, particularly fat. Although total fat intake in Mediterranean populations can be higher than in other regions (ca. 40% of calories), the greater proportion is derived from olive oil and not animals. Increased olive oil consumption is implicated in a reduction in cardiovascular disease, rheumatoid arthritis, and, to a lesser extent, a variety of cancers. Olive oil intake also has been shown to modulate immune function, particularly the inflammatory processes associated with the immune system. Olive oil is a nonoxidative dietary component, and the attenuation of the inflammatory process it elicits could explain its beneficial effects on disease risk since oxidative and inflammatory stresses appear to be underlying factors in the etiology of these diseases in man. The antioxidant effects of olive oil are probably due to a combination of its high oleic acid content (low oxidation potential compared with linoleic acid) and its content of a variety of plant antioxidants, particularly oleuropein, hydroxytyrosol, and tyrosol. It is also possible that the high oleic acid content and a proportionate reduction in linoleic acid intake would allow a greater conversion of α-linolenic acid (18:3n-3) to longer-chain n-3 PUFA, which have characteristic health benefits. Adoption of a Mediterranean diet could confer health benefits in high-risk populations.

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THE MEDITERRANEAN DIET AND HEALTH

The main reason for the interest shown by nutritionists and health professionals in the "Mediterranean diet" relates to epidemiological observations that certain populations living in and around the Mediterranean area have very low incidences of chronic diseases, particularly those with an inflammatory etiology such as heart disease, cancer and rheumatoid arthritis (RA), and that they display greater longevity when compared with northern European and American populations (1–3). Although a great deal has been published that suggests a specific, clearly defined Mediterranean diet exists, this is patently not the case since diets in this region vary significantly between countries. There are, however, strong similarities in the traditional diets from the Mediterranean region that distinguish them from those consumed in northern Europe and North America in that they are largely plant based. The characteristics are not uniform throughout the region but generally include a high consumption of vegetables, fruit, legumes, and grain (in the form of whole-grain bread), a relatively low consumption of meat, particularly red meat, a moderate consumption of dairy products other than cheese, a moderate intake of alcohol (mainly wine at meals), and a relatively high intake of olive oil as the major fat source when compared to Northern European and North American traditional diets (3,4).

This review will focus on the possible health benefits of olive oil, which is the major culinary fat in the classical Mediterranean diet. The total fat intake in this diet can be relatively high, as in Greece with *ca.* 40% of total calories as fat which is higher than in many North American populations, or moderate, as in Italy with *ca.* 30% of calories as fat, but the greater proportion of this fat is derived from olive oil and consequently the diet is rich in monounsaturated FA (MUFA). Fish consumption and the intake of omega-3 PUFA (n-3 PUFA) also vary considerably between different Mediterranean populations, depending mainly on their proximity to the sea. However, the ratio of MUFA to saturated FA is always much greater than in northern Europe and America due to the relatively high olive oil intake in these areas (3,4). The Seven Countries Study (1–5) in the 1950s and 1960s clearly showed that, despite having higher total fat intake (*ca.* 40% of energy), populations in Greece, particularly on Crete where adherence to the traditional diet was most pronounced, have lower disease incidence, particularly cardiovascular disease (CVD), and lower death rates from all causes in middle age than those in northern Europe and North America. This implicated the type, rather than the amount, of dietary fat as a prime causal factor in the etiology of disease, particularly CVD. This is important since positive associations between high fat consumption (*ca.* 40% of energy) and diseases such

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ARA, arachidonic acid; cGPx1, cytosolic glutathione peroxidase; COX, cyclooxygenase: CVD, cardiovascular disease; GPX1, glutathione peroxidase; GPX4, phospholipid glutathione peroxidase; HUVEC, human umbilical vein endothelial cells; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; LOX, lipoxygenase; LT, leukotriene; MUFA, monounsaturated FA; NK, natural killer; PGE_2, prostaglandin E_2; RA, rheumatoid arthritis; ROS, reactive oxygen species; VCAM-1, vascular cell adhesion molecule 1.

as cancer of the colon (6), breast (7,8), prostate (8,9), and ovary (10) as well as atherosclerosis (11) and coronary heart disease (12) are reported. However, the link between total fat intake and cancer of the breast and colon has been refuted (13,14) and, as with CVD, it appears that the type rather than the amount of fat is important in the etiology of some cancers (15). This was supported by evidence from Greece and Israel where the average daily fat intakes are 140 and 100 g, respectively, with higher animal fat (60 vs. 40 g) and vegetable fat (80 vs. 60 g) in the former. However, the lower mortality from breast cancer in Greece than in Israel (16) may be explained by the fact that the vegetable fat in Greece was mainly olive oil whereas that in Israel was mainly sunflower oil (17). Increasing intakes of MUFA as olive oil would be expected to reduce the relative intake of saturated fat as seen in the high MUFA/saturated FA ratios in Mediterranean diets. Replacement of saturated fat with either MUFA or PUFA has been shown to confer health benefits, particularly in relation to CVD (18,19). The relative importance of the MUFA content of the Mediterranean diet to its recognized health benefits will be discussed in this review.

Although the MUFA content of olive oil (*ca.* 85% oleic acid, on average) is important in conferring certain health benefits attributable to this dietary constituent, recent studies have shown that the minor components of this oil may be equally, if not more, important. Although the content of the anti-oxidant vitamin E (α-tocopherol) is low in olive oil compared with corn or safflower oil, the content of other antioxidants such as squalene, simple phenols (e.g., oleuropein, ligstroside-aglycones, tyrosol, hydroxytyrosol, elenolic acid, caffeic acid, apigenin, terpenes, plus numerous others), secoiridoids, and the lignans (e.g., acetoxypinoresinol and pinoresinol) is relatively much higher, particularly in cold-pressed extra virgin olive oil (20). Other dietary sources of phenolic antioxidants are tea, fruit (including grapes, particularly red grapes), and vegetables. Phenols from tea and fruit are water-soluble, whereas those from olive oil are partly lipid-soluble (21). A Mediterranean-type diet rich in olive oil will supply about 10–20 mg of a mixture of these phenols per day (22). These phenolic compounds are absorbed from the diet in humans (*ca.* 55–66% of intake; 23,24) in a dose-dependent manner, appear in plasma, and are excreted in the urine as glucuronide conjugates of mainly tyrosol and hydroxytyrosol. The level of excretion depends on the amount ingested, but only a small fraction of that amount is recovered in urine (23–26). It appears to be impossible to completely eliminate hydroxytyrosol from biological fluids despite strict dietary control preventing its ingestion. This is attributable to the fact that this phenolic compound is also a metabolite (dihydroxyphenylethanol) of dopamine in the body (26). The lipid-soluble phenolic compounds are transported in the lipoprotein fraction of plasma and have been shown to attenuate the oxidation of LDL fractions *in vitro*, and this attenuation has been promulgated as a major factor in the antiatherogenic effects of olive oil (22,27,28).

MECHANISMS OF DISEASE PREVENTION

(i) Cardiovascular disease. As mentioned above, olive oil is the main source of dietary lipids in the Mediterranean regions where mortality and incidence rates for coronary heart disease are the lowest in Europe (1,29,30). This protective effect against heart disease has been attributed, at least in part, to the high content of MUFA in olive oil (*ca.* 85% oleic acid and only 4–5% linoleic acid), which generally results in a concomitant reduction in intake of saturated FA and PUFA. This results in LDL particles that are more resistant to oxidation *in vitro* because of the lower susceptibility to oxidation of MUFA compared with PUFA. In several dietarey studies in humans and experimental animals, virgin olive oil and oleic acid-rich diets that do not contain the phenolic anti-oxidants of olive oil, have been shown to decrease LDL susceptibility to oxidation and to lower LDL-cholesterol levels without affecting HDL-cholesterol; both effects are regarded as antiatherogenic (31–34). Western diets contain an excess of linoleic acid (18:2n-6) (ratio of n-6/n-3 is 15–20:1 instead of the recommended 4–5:1), which has a recognized cholesterol-lowering effect. It is now also recognized that dietary linoleic acid favors such deleterious effects as oxidative modification of LDL-cholesterol, increased platelet aggregation, and suppressed immune function (35). The Cretan diet also contains significant levels of α-linolenic acid (18:3n-3), and the low n-6 PUFA content should allow a greater metabolism of α-linolenic acid to its longer-chain metabolites EPA (20:5n-3) and DHA (22:2n-3), which are the precursors of different, possibly less inflammatory series of prostanoids [III series prostaglandins (PG) and V series leukotrienes (LT) mainly from EPA] than the n-6 PUFA with potential benefits to cardiovascular parameters. This was evident from the greater levels of the latter FA in plasma from Cretan as compared with plasma from Dutch volunteers. Similarly, in the Lyon intervention study, where European volunteers consumed an adapted Mediterranean diet, high plasma concentrations of oleic acid, enhanced 18:3n-3 to 18:2n-6 ratios, and longer-chain n-3 PUFA as well asand higher plasma concentrations of vitamin E and C were observed (35). The intervention group had no sudden deaths (35), suggesting anti-arrhythmic effects similar to those observed for long-chain n-3 PUFA from fish oil (36–38). A previous study showed that high intakes of 18:3n-3 (*ca.* threefold greater than that of control intake at 0.25% of calories vs. 0.755% of calories, respectively) were associated with increased plasma and platelet 20:5n-3 when 18:2n-6 intake was 4.8% of energy but not when it was 8.9% of energy (39). Similar observations were reported by Emken *et al.* (40) in human volunteer studies using isotope tracers. The conversion of deuterated 18:3n-3 to its longer-chain metabolites (EPA and DHA) was reduced by about 50% when dietary intake of 18:2n-6 was increased from 4.7 to 9.3% of energy. This can be explained by the competition between the n-6 and n-3 PUFA families for the desaturation pathway, where an excess of 18:2n-6 will inhibit desaturation of 18:3n-3. In Mediterranean countries, 18:2n-6 in-

take is generally at the moderate level of about 3–4% of dietary energy, thereby allowing adequate 18:3n-3 desaturation. Such effects of high oleic acid intakes have not been given sufficient recognition. Wahle *et al.* (8) observed a marked increase in 22:6n-3 and 20:4n-6, and an even greater decrease in 18:2n-6 of cardiac phospholipids (both PC and PE), when synthetic high-triolein diets were fed to obese Zucker rats. This again suggested an increased desaturation of dietary 18:3n-3 to 22:6n-3 because no fish oil was present in the diets. The beneficial effects of long-chain n-3 PUFA on cardiac arrhythmias in animals are well documented (see above), and it is conceivable that MUFA can elicit similar effects through modulation of desaturation mechanisms by decreasing the competitive inhibition of the high levels of 18:2n-6. This is a mechanism that offers a further explanation for the putative benefits of olive oil consumption, in tandem with 18:3n-3 intake, on CVD. The antiatherogenic effects of olive oil and oleic acid on classical plasma lipid risk factors have been well documented and have already been mentioned in this article.

MUFA-rich, Mediterranean-type diets also elicit significant beneficial, antithrombogenic effects on endothelial products such as von Willebrand factor (vWF), thrombomodulin, Tissue Factor Pathway Inhibitor, and E-selectin, which were all decreased in healthy male volunteers receiving a MUFA-rich diet (41). These decreases correlated with an increase in lag-time for LDL oxidation (41). Precisely how MUFA elicit these effects on endothelial products is not clear, but the mechanism could involve decreased oxidative stimuli and/or changes in FA composition.

In recent years there has been increasing interest in the presence of antiatherogenic and antithrombogenic effects of the minor phenolic components of olive oil as a way to explain the health benefits of the "Mediterranean diet." There is increasing evidence that certain of these compounds, but not all of them, display significant antioxidant activity, can increase the resistance of LDL particles to oxidation *in vivo* and *in vitro* (20–22,27), and inhibit platelet aggregation *in vitro* (42). Since atherogenesis and CVD are increasingly being regarded as an oxidative-inflammatory stress disorder (43), these dietary phenolic compounds offer another explanation for the observed cardiovascular health benefits, as well as other oxidative or inflammatory stress-induced diseases (see following discussion), of dietary olive oil. Phenolic compounds from virgin olive oil and red wine elicit protection in animal models against inflammation, inhibit vascular smooth muscle cell migration, and, at the cellular level, enhance the expression and activity of endothelial nitric oxide synthase (44–46).

Leukocyte/monocyte adhesion to the endothelium and their extravasation into the vessel wall is a very early stage of the atherogenic process leading to vascular disease and the first indication of endothelial dysfunction. The mechanism to explain this process appears to be predominantly the oxidative stress and/or inflammatory cytokine-induced increase in expression at the endothelial cell surface of specific endothelial-leukocyte adhesion molecules that tether the circulating cells prior to their invasion of the intima of the vessel wall (47,48). Circulating cancer cells utilize the same process prior to the development of metastatic secondary loci in various tissues (49).

The expression of these adhesion molecules in cells at the transcriptional and translational level (mRNA and protein) as well as the activity level appears to be largely regulated by redox-sensitive mechanisms in the cells, with enhanced redox state leading to decreased expression (50–53). n-3 Long-chain PUFA from fish, despite their reported enhancement of LDL oxidation *in vitro*, decrease the expression of cytokine-induced adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), E-selectin, and P-selectin that are present on activated endothelial cells, and leukocytes/monocytes at the mRNA, protein, and activity level (54–59). A possible explanation for the effect of n-3 PUFA on adhesion molecule expression is the observation that these FA can up-regulate the expression and activity of the redox enzymes glutathione peroxidase (GPX1) and phospholipid glutathione peroxidase (GPX4) when compared with the n-6 arachidonic acid (ARA; 20:4n-6), a process that would be expected to decrease expression of the adhesion molecules (51). Inhibition of adhesion molecule expression in human umbilical vein endothelial cells (HUVEC) by CLA could also be due to the observed up-regulation of these redox enzymes when these FA are present (60,61).

Phenolic compounds from both olive oil and red wine such as oleuropein, tyrosol, hydroxytyrosol, apigenin and resveratrol at concentrations of *ca.* 30 μM can significantly attenuate the inflammatory cytokine-induced activation of endothelial cells and the transcriptional/translational up-regulation of adhesion molecules like ICAM-1, VCAM-1, and E-selectin by *ca.* 40–70% (61–63) and decrease monocyte cell adhesion to activated endothelial cells by *ca.* 20–30% (63). Surprisingly, a total phenolic extract of olive oil at concentrations from 10 to 250 μM actually increased the expression of ICAM-1 and E-selectin mRNA in HUVEC by about 25 to 550% in the presence of 10 U/mL interleukin-1β but elicited no effect on expression without the cytokine (61). The explanation for this is not clear, since hydroxytyrosol, apigenin and, to a lesser extent, tyrosol at similar concentrations inhibited the mRNA expression of these adhesion molecules (61). The lesser effect of tyrosol was to be expected since this phenolic compound is less of an antioxidant than hydroxytyrosol (20).

The effects of olive oil and red wine phenolics on gene expression of adhesion molecules are mostly determined individually, and little is known of any interactions or synergisms between these compounds and other components of the diet. We have investigated the interaction of the polyphenolics from green tea, quercetin, and kaempferol, and have observed a significant synergism between these compounds and n-3 PUFA in inhibiting the cytokine-induced expression of adhesion molecules on HUVEC. The presence of either n-3 PUFA or phenolics alone resulted in a *ca.* 50% inhibition of expression compared with control, but this was not as great an inhi-

bition as when the compounds were present in combination, where a further *ca.* 50% inhibition of the individual effects was observed (64). This is a reminder that the effects of dietary components should not be investigated or interpreted in isolation and that diets are a complex mixture of compounds that can have surprising interactions. Another case in point is the observation that, when phenolics are extracted together as a mixture from olive oil, they appear to have the opposite effect to the individual phenolic compounds. Whether similar interactions between n-3 PUFA and the phenolics from olive oil also occur is presently being investigated in our laboratory.

(ii) Cancer. The overall incidence of cancer is significantly lower in countries adhering to or partly adhering to the traditional Mediterranean diet when compared with northern Europe and North America, but differences are not as dramatic as seen with CVD (65,66). The differences are largely due to the lower incidence of colorectal cancer and cancer of the breast, prostate, and endometrium in these regions. The etiology of these specific cancers appears to have a strong dietary element and includes low intakes of fruit, vegetables, and whole-grain products and relatively high intakes of animal products (meat and fat) (3,4,67). It has been estimated that about 25% of colorectal cancer, 15% of breast cancer, and 10% of prostate, endometrial, and pancreatic cancers could be prevented if the populations of the more developed northern European countries and North America adopted a traditional Mediterranean type diet (3,4).

(iii) Cell mechanisms affected by diet. Although the traditional Mediterranean diet, particularly the intake of fruit, vegetables, and olive oil, is capable of reducing the incidence of various cancers, the cellular mechanisms by which this is achieved are not clearly understood. It is conceivable that the lower intake of linoleic acid and higher intakes of oleic and α-linolenic acids could play an important role in prevention/amelioration of certain cancers. In animal models of various cancers, the feeding of high levels of linoleic acid enhanced tumor growth, development, and metastasis whereas feeding long-chain n-3 PUFA from fish (EPA and DHA) elicited the opposite effect (68). As already mentioned, the high oleic acid intake and lower linoleic acid intake in olive oil-based Mediterranean diets would allow greater metabolism of 18:3n-3 to the longer-chain n-3 derivatives found in fish oils (EPA and possibly DHA) and could possibly elicit a similar effect to that of fish oil in reducing tumor growth and metastasis (68–70).

The beneficial effects of these n-3 PUFA in both vascular disease and animal models of cancer (and possibly in other immune/inflammatory-based diseases) appear to reside in their ability to attenuate the effects of oxidative and inflammatory processes, partly by inhibiting the expression of inflammatory cytokines at the level of gene transcription and partly by ameliorating the actions of these cytokines on cells through down-regulation of the cellular signal mechanisms (kinases, transcription factors: see following paragraphs) and inhibition of inflammatory eicosanoid formation (mainly reduction of PGE_2). The excessive production of PGE_2 is a characteristic of many tumors and is apparently a mechanism to enhance tumor growth and development (69). Inhibition of PGE_2 formation by inhibition of the cyclooxygenase-2 (COX-2) enzyme with aspirin has been shown to reduce colon cancer significantly (71). Reduced incidence of certain cancers in populations consuming Mediterranean-type diets with high 18:3n-3 and oleic acid but low 18:2n-6 contents compared with the average European and North American diets could be associated with lower PGE_2 production in tissues in response to an oxidative stress or/and an inflammatory insult and a lower capacity to produce the cytokines responsible for such an insult.

Our group has shown that the important constituents of olive oil, namely, oleic acid and phenolics like hydroxytyrosol, tyrosol, caffeic acid, and apigenin (the latter is present only as a minor component in olive oil but is a constituent of other edible plant species in the diet) can significantly attenuate the gene expression of eicosanoid-synthesizing enzymes such as 5-lipoxygenase (5-LOX) and its co-enzyme 5-LOX-activating protein by 20–25%. The most marked inhibition was observed with apigenin followed by hydroxytyrosol and oleic acid, with tyrosol and caffeic acid having little or no effect. The LOX enzyme system, which is responsible for the production of pro-inflammatory LT formation from n-6 PUFA, was affected to a greater extent than the COX system (COX-1 and -2), which produces the pro-inflammatory prostaglandins from n-6 PUFA (72). However, the formation of PGE2 was significantly decreased by hydroxytyrosol (and to a lesser extent by tyrosol and caffeic acid) in prostate cancer cells. This suggests that the olive oil components may have an effect on the gene expression of the LOX pathway and that the observed phenolic effects on PGE_2 reduction are most likely posttranscriptional effects. It also indicates the possibility that this LOX pathway is more important for enhancing the development and progression of tumors than hitherto thought. Increasing EPA (20:5n-3) availability in cells may alter the pro-inflammatory eicosanoid profile derived from ARA (20:4n-6) by decreasing PGE_2 synthesis and increasing the formation of three series PG and five series LT by substituting for ARA in the enzyme reactions (73). Since the high oleic acid, relatively high α-linolenic acid, and relatively lower linoleic acid contents in Mediterranean diets favor an increased conversion of 18:3n-3 to 20:5n-3 (and to a lesser extent 22:6n-3) and since this diet also incorporates significant amounts of fish, it is possible, but not proven, that the lower cancer incidence in these countries is related in part to reduced inflammation and eicosanoid production in response to specific stimuli. This is supported by epidemiological evidence of reduced incidence of certain types of cancer (large bowel, breast, prostate) in some populations with relatively high fish consumption (74).

Oxidative stress and an inability to counteract its consequences were suggested to be a component in the etiology of a number of cancers. Hydrogen peroxide and reactive oxygen species (ROS) formed during metabolic processes in the cell can result in damage to cellular components including lipids,

proteins, and, most importantly, DNA (75). These actions of ROS can result in lipid peroxide formation, cell dysfunction, DNA damage, and cell death. DNA damage can result in mutations and impairment of cell-cycle regulation, leading to the uncontrolled cell proliferation that is a characteristic of tumor growth (75). The relatively high intake of antioxidants in the traditional Mediterranean diet would tend to attenuate oxidative stress and reduce its deleterious cancer-promoting effects (3,4).

Our group showed that DNA damage, induced by H_2O_2 treatment of prostate cells, was decreased by olive oil phenolics and by increasing the amount of oleic acid presented to cells. The most effective compound was hydroxytyrosol, followed by caffeic acid, with tyrosol, as expected from its lower antioxidant capabilities (20), being the least effective but still affording protection at higher concentrations. The amelioration of DNA damage by these phenolics increased with their increasing concentration, with beneficial effects observed in physiological concentration ranges (10–50 μM) . However, the greatest effect of each compound was observed at relatively high and possibly nonphysiological concentrations (>200 μM) (76). The only phenolic eliciting significant inhibition of hydroperoxide formation in these cells, a biomarker of ROS-induced lipid peroxidation, was hydroxytyrosol, and the effect was not influenced by increasing its concentration. Tyrosol, in contrast, elicited a slight but significant increase in hydroperoxide production, whereas caffeic acid produced a nonsignificant, small decrease in this marker of ROS damage (76). The effects on hydroperoxide formation by the olive oil phenolics were mirrored by their effects on the gene expression of the important intrinsic redox enzyme, cytosolic glutathione peroxidase (cGPX1). The abundance of stable mRNA of cGPX1 was significantly and markedly decreased by hydroxytyrosol at all concentrations from 10 μM to nonphysiological 250 μM. Caffeic acid was less effective than hydroxytyrosol but still decreased expression significantly, whereas tyrosol elicited an increase in expression. Similar but less marked effects of these phenolics were observed on GPX4 mRNA expression. This enzyme is responsible for reducing hydroperoxides formed by ROS action in cell membranes, particularly mitochondrial membranes (76). The attenuation of the hydroperoxide formation and redox enzyme expression by phenolics, particularly hydroxytyrosol, is a strong indication that these extrinsic dietary compounds are functioning as antioxidants and decreasing the requirement for the induction of the intrinsic antioxidant enzymes. However, an induction of the expression of these cell antioxidant enzymes by dietary components such as n-3 PUFA also has been suggested to play a beneficial role in the prevention of cell dysfunction and disease states so long as the inducing stimuli do not overwhelm the cells' capacity for up-regulating these enzymes. (77). It has also been suggested that an individual's inability to up-regulate these intrinsic antioxidants, possibly due to polymorphisms (SNPs) in gene expression, may explain individual susceptibilities to cancer within populations, particularly if the intake of dietary antioxidants is low (77).

Although the efficacy of the Mediterranean diet in reducing incidence of cancer is not so marked as the reduction in CVD, it is nevertheless significant and is strongly correlated with the intake of olive oil on an epidemiological basis (2–4). Whether these effects are entirely due to the overall lower oxidative potential of classical Mediterranean diets or to specific components such as the phenolics present in the oil or antioxidants derived from other components of the diet is not clear and warrants further research. Similarly, it is not clear whether the hydroxytyrosol or tyrosol from olive oil is capable of augmenting the effects of specific FA in the diet as observed for DHA and the polyphenolic flavonoid quercetin (61). These aspects of olive oil are currently being investigated in our laboratory.

IMMUNE FUNCTION AND RA

Animal studies have shown that dietary olive oil has a significant suppressive effect on lymph node lymphocyte proliferation *ex vivo* in response to the T-cell mitogen Concanavalin A in comparison with diets rich in coconut oil or safflower oil or diets low in total fat. The effect of the olive oil diets was found to be similar to diets containing either fish oil or evening primrose oil (78). To determine whether the effects of olive oil feeding were due to components other than the high oleic acid content of these oils, these authors investigated the effects of diets containing high-oleic acid sunflower oil and compared the findings with the diets just described. Feeding either olive oil or high oleic acid sunflower oil significantly decreased lymphocyte proliferation by *ca.* 20% compared with the other diets (79). This suggested that these specific effects of the olive oil diets on lymphocyte proliferation were due largely to their content of oleic acid and not to the content of antioxidant phenolic compounds. This suppression of lymphocyte proliferation does not occur in middle-aged male volunteers consuming highly refined olive oil (lacking the phenolic antioxidants) (80). These contrasting effects may be explained by the fact that the rat diets were extreme (30% energy) compared with human diets (18% energy) or by species differences (81). Similar inhibitory effects on natural killer (NK) cell activity were observed in rats fed a high olive oil diet (20% by weight) or a diet high in oleic acid (30% of total energy), again suggesting effects are due to the oleic acid content of the diet (79). This was supported by the findings of a significant linear relationship between oleic acid content of a diet and NK cell activity (79). Again, the effects of oleic acid on immune function in healthy middle-aged male volunteers contrasted with findings in rats, and no significant effects on NK cell activity were evident after 1 mon on the diet although slight suppression was observed after 2 mon on the diet (80).

Expression of various adhesion molecules in the blood vascular system is up-regulated by immune-inflammatory stimuli and is a significant component of the thromboatherogenic process in animals and humans (82). In a dietary study in animals, the expression of adhesion molecules [ICAM-1, LFA-1 (lymphocytle function antigen-1) and CD2 (cluster of

differentiation 2)] on rat spleen lymphocytes was decreased by both olive oil and fish oil feeding (83). Similarly, after 2 mon on a high oleic acid diet, a significant reduction in ICAM-1 was observed in middle-aged male volunteers (80). No changes were found with the control diet, again suggesting that oleic acid content of the diet has specific effects on inflammatory responses. It is not clear whether the effects observed with high oleic acid diets are due to changes in other FA on a proportional basis. Increasing oleic acid will decrease the relative proportion of other FA consumed. A decrease in linoleic acid, as previously mentioned, could allow greater conversion of α-linolenic acid to long-chain n-3 PUFA. This might explain the similarities between olive oil or high-MUFA diets and fish oil diets (already discussed). The other possibility is that high-MUFA diets like the Mediterranean diet are less likely to induce oxidative stress, which is the stimulus for adhesion molecule up-regulation in the vascular system. The n-3 PUFA are known to enhance the expression and activity of intrinsic redox enzymes (GPX1 and GPX4) significantly, and this enhancement could be pivotal in their ability to reduce adhesion molecule expression since these enzymes are involved in the regulation of the nuclear factor κB (NF-κB) oxidative stress-activation pathway (51,56).

High olive oil diets (*ca.* 20% by weight) also attenuated the response of the immune system to endotoxins and the anorexia induced by increased production of the inflammatory cytokine tumor necrosis factor-α and the graft vs. host responses in animals (83,84). Again, the results indicate a similar effect of olive oil to that of fish oil in the diet. The expression of adhesion molecules in popliteal lymph nodes of animals following graft vs. host responses was significantly lower in animals fed olive oil or fish oil compared with low-fat or coconut fat diets (83). It appeared that olive oil was able to modulate *in vivo* responses involving B cells but not cytotoxic T lymphocytes whereas fish oil could modulate both responses (83).

Anti-inflammatory effects of olive oil consumption have also been reported for RA. In a Greek population, consumers of high amounts of olive oil (almost every day throughout life) were four times less likely to develop RA than those subjects who consumed the oil less than six times per month (85). Interestingly, in the light of similar effects of fish oil and olive oil in other studies, fish oil was without significant effect in this study (85). This contrasts with some other studies that showed a beneficial effect of dietary fish oil in reducing the symptoms of RA. Kremer *et al.* (86) showed significant attenuating effects of both fish oil and olive oil on RA and the production of the inflammatory cytokine IL-1, with the former eliciting the greater benefit in reducing both IL-1β production and the symptoms of RA. In conclusion, olive oil apparently is able to attenuate the activity of certain immune cells in animals, albeit to a lesser extent than dietary fish oil. Some evidence suggests that the effect of olive oil is primarily due to its monounsaturated oleic acid content, but this requires verification. Studies with human volunteers do not show the same attenuating effects of MUFA/olive oil on immune function and could reflect the high content of these components in the animal diets (*ca.* 20% by weight). The effect of high MUFA/olive oil on adhesion molecule expression in the blood vascular cells in both animals and humans could be an important effect of these dietary components, as increased expression of these molecules appears in a number of inflammatory diseases including CVD, cancer, and RA. Clearly, a Mediterranean diet with high oleic acid and relatively high content of a-linolenic as well as the various antioxidant components in both olive oil and other plant foods (mono- and polyphenolics) presents a low potential for oxidation in the body. This could be an additional factor in the benefits elicited by such a diet since the oxidative-inflammatory processes are associated with the most common diseases in industrialized societies. Adoption of a diet similar to the traditional Mediterranean diet with its high intakes of fruit, vegetables, whole-grain products and olive oil and low intakes of refined carbohydrates and red meat would also reduce the disease risk and enhance the life expectancy of northern European populations.

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