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The Effect of Minor Constituents of Olive Oil on Cardiovascular Disease: New Findings

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There has been much interest regarding the components that contribute to the beneficial health effects of the Mediterranean diet. Recent findings suggest that polyphenolic compounds found in olive oil are endowed with several biologic activities that may contribute to the lower incidence of coronary heart disease in the Mediterranean area.

Introduction

The Mediterranean diet, which is high in fruits, vegetables, grains, and legumes and relatively low in meat, is associated with a lower incidence of coronary heart disease and, possibly, cancer.[1–3] Dietary fats are significant environmental determinants of atherosclerosis, and olive oil is the fat of choice of the Mediterranean area, where it is consumed in large amounts. Olive oil is rich in oleic acid (18:1n-9), which is also the most abundant monounsaturated fatty acid in all diets and tissues. The high intake of oleic acid from olive oil and the concomitant low intake of saturated fat, compared with the larger consumption of saturated fat in Northern Europe and the United States, has been proposed as an important factor in the favorable health effects of the Mediterranean diet.

Olive Oil

Olive oil is obtained from the olives or drupes (fruits) of *Olea europea*, a tree that is best grown between the 30th and 45th parallels. Mediterranean countries produce more than 95% of the world's olive oil supply, 75% of which comes from the European Union. Consumption of olive oil is increasing in non-Mediterranean areas such as the United States (where olive oil imports exceed 100,000 metric tons/year), Canada, Russia, Australia, and Japan, because of the growing interest in the Mediterranean diet.

Olive oil has a unique fatty acid composition: its oleic acid content ranges from 56% to 84%, whereas linoleic acid (18:2n-6) constitutes 3–21%.[4] Although the major effects of high monounsaturated fat intakes on serum cholesterol are generally attributed to the associated replacement of saturated fatty acids in the diet,[5,6] some studies[7] have attributed a direct but modest cholesterol-lowering effect of monounsaturated fat alone when it equicalorically replaces carbohydrate intake. Furthermore, monounsaturated fat increases the levels of the protective high-density lipoprotein (HDL) cholesterol more than polyunsaturated fat does when these two classes of fatty acids replace carbohydrates in the diet.[7] There is evidence that the optimal dietary fat should be rich in monounsaturated fatty acids, such as oleic acid, low in saturated fatty acids, and provide adequate amounts of polyunsaturated fatty acids. It should also be noted, however, that oleic acid is one of the predominant fatty acids in widely consumed animal foods, such as poultry and pork; thus, the percentage of oleic acid in the Mediterranean diet is only slightly higher than that of other kinds of Western diets, such as the American diet.[8,9]

Extra-virgin olive oil contains a variety of minor components that produce its particular aroma and taste (Table 1). In fact, although most vegetable oils are extracted from seeds by solvents, olive oil is obtained from the whole fruit by means of physical pressure without the use of chemicals. During this procedure, all of the components of the drupe are transferred to the oil, therefore retaining all of the organoleptic properties of olives. It should be noted that fruits and vegetables are continuously exposed to environmental stresses, including ultraviolet radiation and relatively high temperatures, and need a variety of compounds, such as antioxidants of different origin, to preserve their integrity.

The polyphenolic fraction of olive oil amounts to 50–800 mg/kg, depending on several key factors, such as the cultivar, soil, degree of ripeness of the drupes, and the way the oil is produced and stored.[10–16] Many phenolic components have been identified and can be conveniently classified into two major subclasses: simple and complex (hydrolyzable). The former includes hydroxytyrosol (3,4-

Table 1. Nonglyceride Components of Olive Oil

Hydrocarbons	Anthocyanins
Nonglyceride esters	Hydroxy- and dihydroxyterpenic acids
Tocopherols	Sterols
Alkanols	Phenolic constituents
Flavonoids	Phospholipids

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dihydroxyphenylethanol), tyrosol, and phenolic acids such as vanillic and caffeic acid. The latter includes tyrosol and hydroxytyrosol esters, oleuropein and its aglycone, and several other molecules yet to be identified. Oleuropein (Figure 1) is responsible for the bitter taste of olives and for the browning of the olive skin. Its levels slowly taper off during the late days of the ripening season, yielding several simpler molecules that build up the full, fruity taste of the oil.

The absolute concentrations and relative proportions of olive oil's minor components are characteristic of each batch of oil, allowing for identification of the area of production and potential adulterations. Because the olive paste is continuously hosed with warm water during the milling process in order to remove foreign bodies and debris, a high amount of "waste water" is produced and subsequently discarded. Some of the polyphenols contained in the olives end up in the water phase, according to their partition coefficient. Recently, we performed a series of experiments in which we demonstrated that waste waters have powerful (in the parts per million range) antioxidant activity and might therefore be recovered and employed in preservative chemistry as an inexpensive, and as yet unused, source of natural antioxidants.[17]

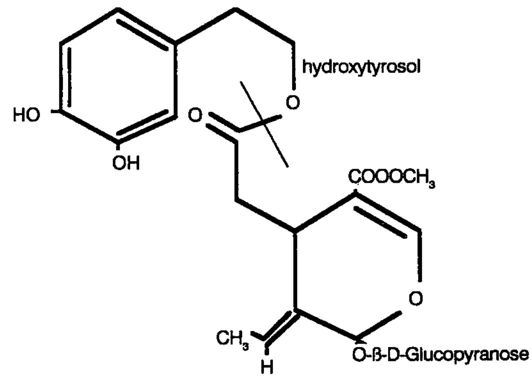

Figure 1. Chemical structure of oleuropein. Hydroxytyrosol is obtained by enzymatic cleavage where shown.

Biologic Activity of Olive Oil Polyphenols

The availability of pure compounds, either from commercial sources or extracted and purified from olive oil, led us to investigate the antioxidant properties of olive oil polyphenols in models of chemically induced oxidation of low-density lipoproteins (LDL). This model was chosen because of its particular relevance with respect to the onset of atherosclerosis.[18] We measured several indices of lipid and protein oxidation and employed different means of oxidative stress induction. Some of the results are reported in this review.

Vitamin E is the most important lipophylic radical-trapping antioxidant in LDL cholesterol. The addition of copper sulfate (5 μM) to LDL leads to a rapid loss of vitamin E, which disappears within 30 minutes. Preincubation of LDL with olive oil phenolics, such as oleuropein or hydroxytyrosol, at a 10^{-5} M concentration reduced the fall in vitamin E levels so that after 30 minutes of incubation with $CuSO_4$, 80% of the initial vitamin E was still present (Figure 2). By sparing the LDL endogenous antioxidants, olive oil polyphenols prevent the formation of other products of lipid peroxidation,

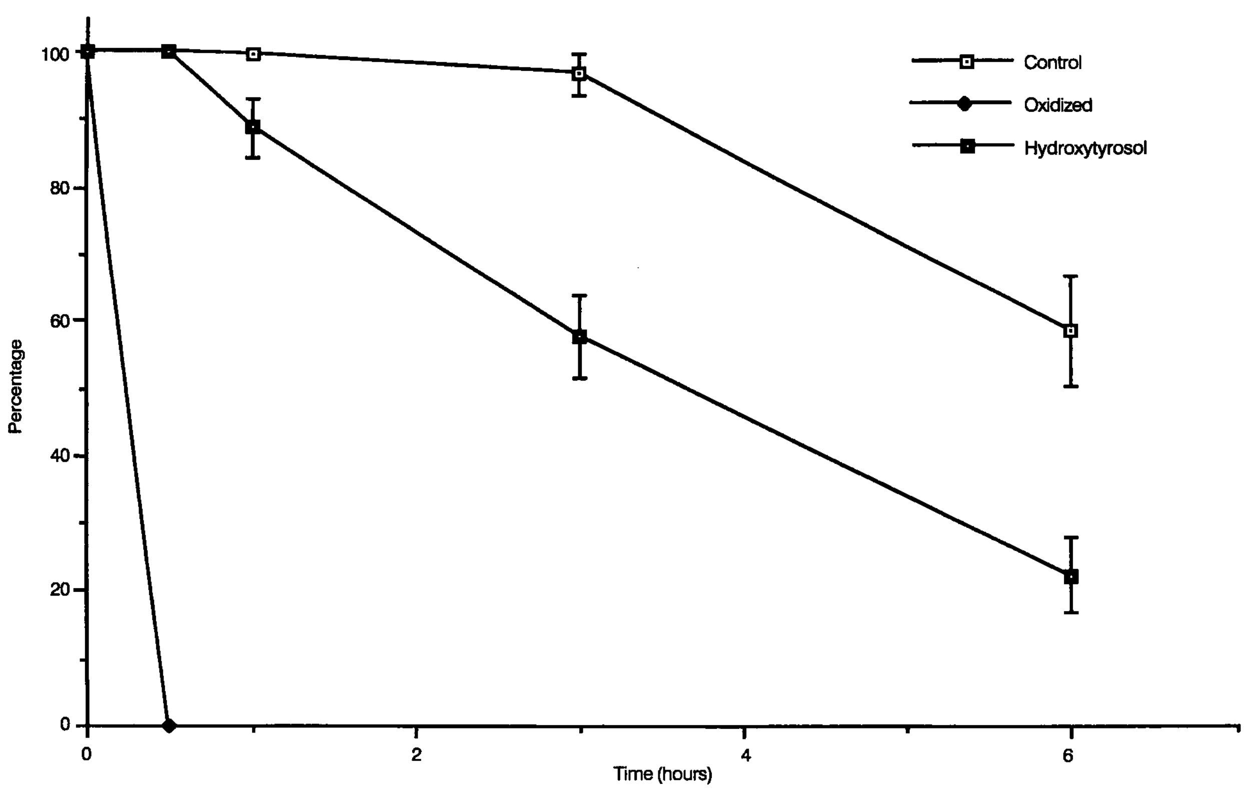

Figure 2. Vitamin E levels in $CuSO_4$-oxidized LDL.

such as isoprostanes, malondialdehyde, and lipoperoxides, keeping their levels close to basal throughout the incubation.

Because the outer layer of LDL is mostly made of phospholipids, polyunsaturated fatty acids are readily exposed to oxidation. Accordingly, the degree of oxidation of fatty acids follows their degree of unsaturation: after 6 hours of incubation with $CuSO_4$, linoleic acid levels were 68% of the initial values, whereas docosahexaenoic acid levels were 25%. Oleuropein and hydroxytyrosol prevented the loss in polyunsaturated fatty acids, the levels of which were close to the initial ones at the end of the 6-hour incubation period (Figure 3).

Protein modification was also prevented by olive oil polyphenols, as shown by the reduced formation of fluorescent adducts between short-chain aldehydes and lysine (Figure 4). Preservation of apoprotein integrity reduces its interaction with the macrophage scavenger receptor, thus limiting the uptake of LDL through this route.

To investigate the mechanisms of action of olive oil polyphenols, experiments in which the oxidative stress was triggered by a metal-independent system were performed. The kinetics of conjugated diene formation showed that although hydroxytyrosol completely inhibited $CuSO_4$-induced oxidation, it doubled the lag phase when horseradish peroxidase/hydrogen peroxide was employed

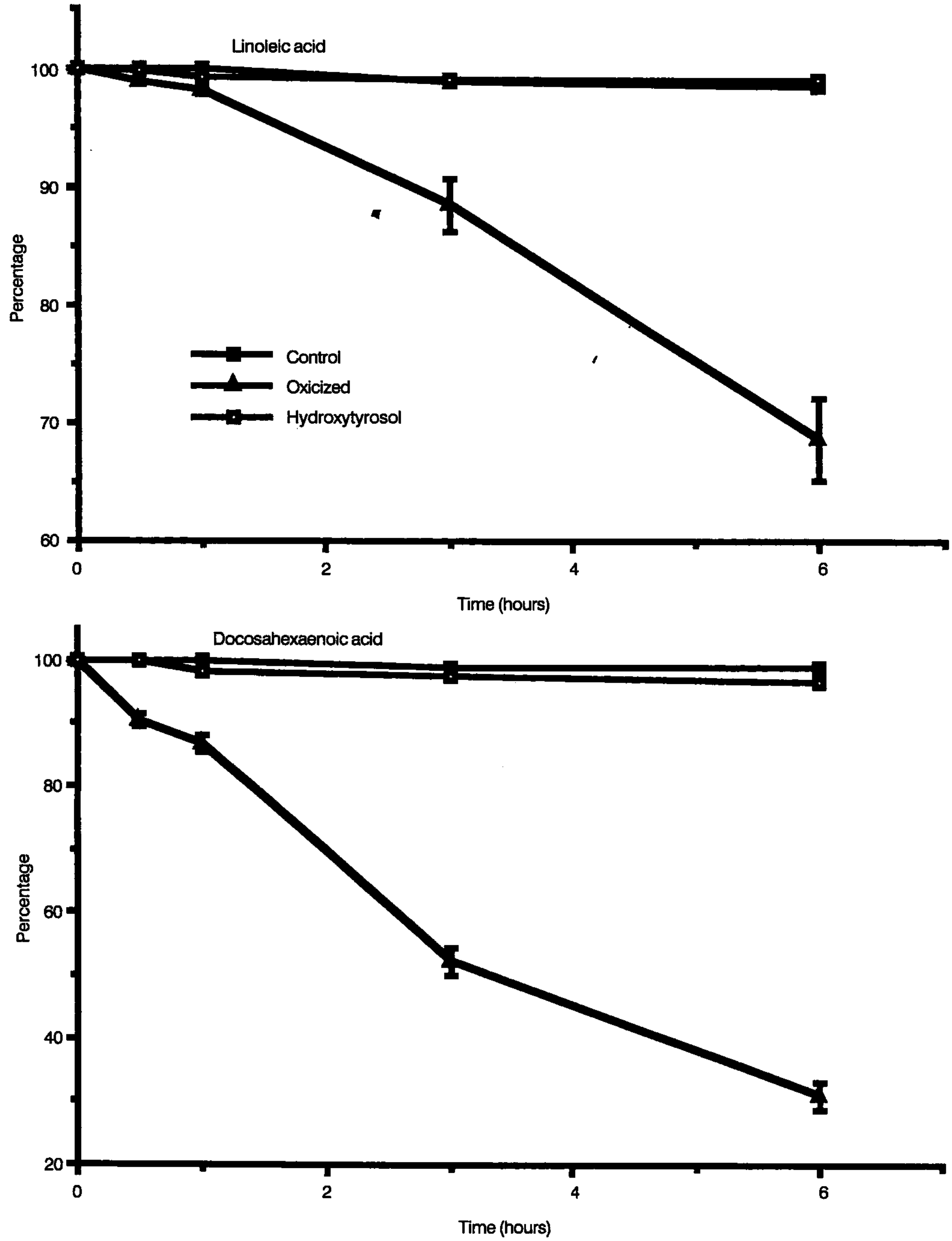

Figure 3. Selected polyunsaturated fatty acid levels in $CuSO_4$-oxidized LDL.

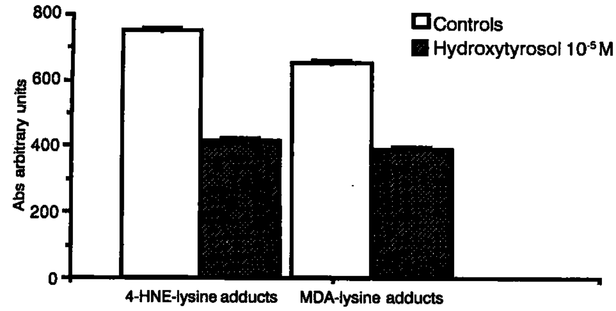

Figure 4. Short-chain aldehydes–lysine adducts formation in $CuSO_4$-oxidized LDL.

(Figure 5). These data suggest that hydroxytyrosol exerts antioxidant activity both by chelating free metal ions and by scavenging newly formed free radicals. The radical-scavenging properties of olive oil polyphenols were confirmed by the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) quenching test, in which oleuropein and hydroxytyrosol proved to be equally or more effective than other established antioxidants such as vitamins C and E and butylated hydroxytoluene (BHT) (Table 2).

Other biologic activities of olive oil phenolics include inhibition of platelet aggregation, reduced eicosanoid production by activated leukocytes,[19] and increased nitric oxide production by endotoxin-challenged mouse macrophages.[20,21] The former activities may modulate thrombus formation and inflammation, respectively, and the latter probably plays a role in host defense against parasites.

Table 2. Free Radical–scavenging Activity According to DPPH Quenching Test

Compound	EC_{50}
Vitamin C	1.31×10^{-5}
Vitamin E	5.04×10^{-6}
BHT	1.05×10^{-4}
Hydroxytyrosol	2.60×10^{-7}
Oleuropein	3.63×10^{-5}

A 15 µM ethanolic solution of DPPH was added to the compounds under investigation. After 15 minutes of incubation, absorbance was read at 517 nm.

Conclusions

The contribution of excessive free radical formation to the onset of certain pathologies may require a high dietary intake of fruits and vegetables, i.e., food with a substantial proportion of antioxidant vitamins, flavonoids, and polyphenols. The beneficial effects of the Mediterranean diet on the cardiovascular system so far have been attributed mostly to its unique lipid profile, but the contribution of natural antioxidants to this effect should also be taken into consideration. The presence of nonsaponifiable components may partly explain the beneficial effects of the Mediterranean diet, despite the fact that olive oil consumption may provide an oleic acid intake similar to that attained with other kinds of diets.[8,9] In this respect, the choice of extra-virgin olive oil, which has the highest content of polyphenols, allows for a higher consumption of antioxidants (approximately 30–50 mg/day),[22,23]

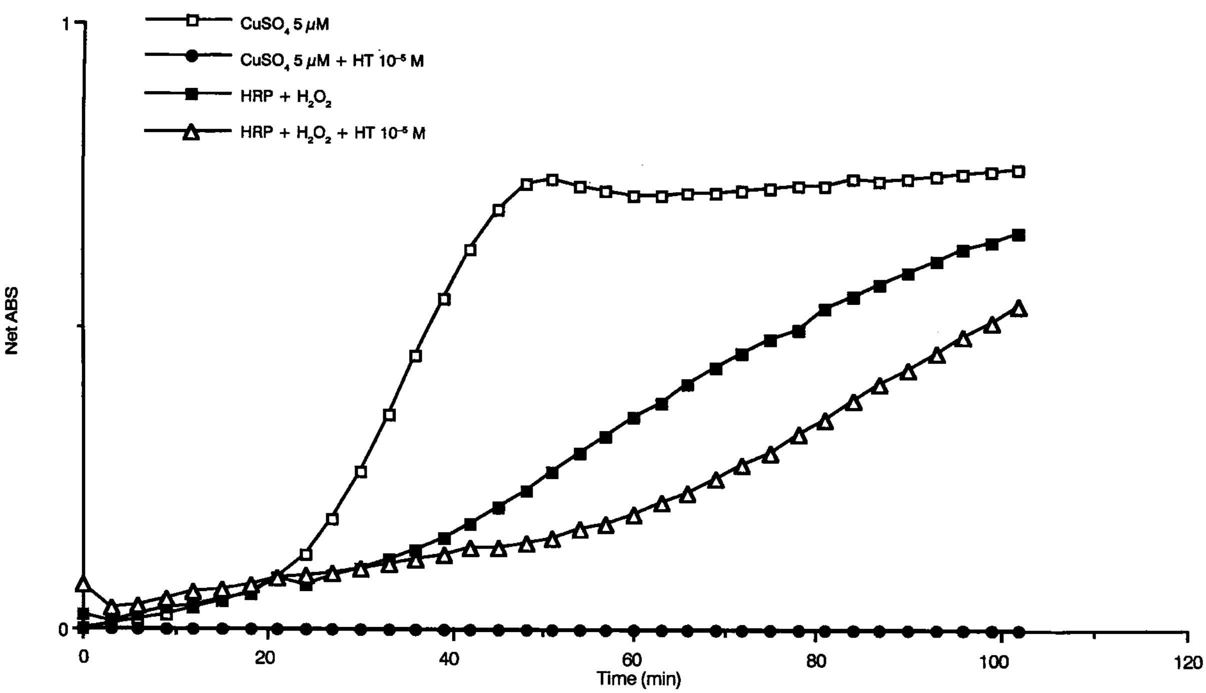

Figure 5. Conjugated diene formation in $CuSO_4$-oxidized and horseradish peroxidase/hydrogen peroxide–oxidized LDL.

Table 3. Biologic Effects of Olive Oil Phenolics

- Inhibition of LDL oxidation
- Free radical scavenging
- Inhibition of platelet aggregation
- Reduced TXB2 and LTB4 production by activated leukocytes
- Enhanced nitric oxide generation by lipopolysaccharide-challenged murine macrophages

which are endowed with potent biologic activities (Table 3).[17,19,20,22–24]

Debate is still going on as to whether flavonoids and polyphenols are actually absorbed and exert their actions in vivo. Development of appropriate methodologies would address this important issue, but there is evidence that a diet rich in olive oil phenolics decreases LDL oxidizability in laboratory animals.[25] Furthermore, absorption and disposition of flavonoids have been reported in humans, although studies on this are still scarce.[26,27]

Although this review focuses on a specific subfraction of olive oil, the polyphenols, the large body of evidence that indicates a beneficial role of olive oil as a whole should not be overlooked. Epidemiologic studies, including the frequently cited Seven Countries Study, as well as intervention trials have reported on the healthful effects of the Mediterranean diet and olive oil. It is, however, difficult to single out an individual component of such a diet and correlate it with the observed lower incidence of coronary heart disease and certain cancers. It is noteworthy that in Crete, where fat consumption provides up to 40% of total calories and is almost totally derived from olive oil, the incidence of coronary heart disease is very low. The biologically relevant properties of olive oil phenolics described in this review provide new insights on the possible mechanisms by which high-quality olive oil contributes to lower mortality from coronary heart disease.

It therefore seems appropriate to suggest the adoption of a diet rich in fruits and vegetables and in which high-quality olive oil represents the highest proportion of fat, because the beneficial effects of the Mediterranean diet may be due, in part, to its high content of natural antioxidants.

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