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Olive-oil Phenolics and Health: Potential Biological Properties

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Extra virgin olive oil, the primary source of oil in the Mediterranean diet, differs significantly in composition from dietary lipids that are consumed by other populations. The several minor constituents of virgin olive oil include vitamins such as alpha- and gamma-tocopherols (around 200 ppm) and beta-carotene, phytosterols, pigments, terpenic acids, flavonoids, squalene, and a number of phenolic compounds, such as hydroxytyrosol, usually grouped under the rubric "polyphenols". The antioxidant and enzyme-modulating activities of extra virgin olive oil phenolics, such as their ability to inhibit NF-kB activation in human monocyte/macrophages has been demonstrated *in vitro*. There is also solid evidence that extra virgin olive oil phenolic compounds are absorbed and their human metabolism has been elucidated. Several activities that might be associated with cardiovascular protection, such as inhibition of platelet aggregation and reduction of plasma rHcy have been demonstrated *in vivo*. The biologically relevant properties of olive phenolics are described, although further investigations in controlled clinical trials are needed to support the hypothesis that virgin olive oil consumption may contribute to lower cardiovascular mortality.

Keywords: extravirgin olive oil, phenolic compounds, Mediterranean diet, hydroxytyrosol.

Numerous epidemiological studies have shown that the incidence of coronary heart disease (CHD) and certain cancers, for example breast and colon cancers, is lowest in the Mediterranean basin [1]. It has been suggested that this is largely due to the protective dietary habits of this area [1,2]. The traditional Mediterranean diet, rich in fruit, vegetables, fish, and whole grain, is thought to promote good health and longevity. Olive oil, the primary oil source of this diet, differs significantly in composition from dietary lipids that are consumed by other populations. The formulation of an antioxidant/atherosclerosis hypothesis stimulated experimental and epidemiological studies on the possible role of antioxidants, including olive oil phenolics, in the protection from CHD observed in the Mediterranean area. Included among the minor constituents of virgin olive oil are vitamins such as α- and γ-tocopherols (around 200 ppm) and β-carotene, phytosterols, pigments, terpenic acids, flavonoids, squalene, and a number of phenolic compounds, usually grouped under the rubric "polyphenols" [3].

Epidemiological studies: From a nutritional point of view, the choice of a phenol-rich olive oil contributes to the dietary intake of biologically-active compounds in quantities that have been correlated with a reduced risk of developing CHD [4]. Indeed, the use of extra-virgin olive oil as the principle source of dietary oil instead of animal fat, in addition to providing a considerable amount of oleic acid, provides an intake of bioactive compounds with potential healthful effects, as described above. It also appears that the intake and interaction of several "micronutrients" provided by a healthy diet, such as that in use in the Mediterranean area during the mid-1940s, is likely to be the link that affords protection from such pathologies [5]. In turn, the answer to the current debate on the efficacy of antioxidant supplements is likely to be found in the adoption of a Mediterranean-style diet, in which the abundance of bioactive, functional compounds provided by fruits, vegetables, wine, and olive oil grants a higher protection toward reactive oxygen species (ROS)-induced diseases.

***In vitro* studies:** The lower incidence of CHD observed in the Mediterranean area [1] lead to the hypothesis that olive oil phenolics exert a protective effect with respect to chemically-induced oxidation of human LDL, which is one of the initial steps in the onset of atherosclerosis [6].

Results obtained on human LDL demonstrate that catechol-like compounds present in virgin olive oil inhibit the formation of lipid oxidation products in a dose dependent manner and are effective at a concentration lower than that of pure tyrosol, a phenolic component of the oil, and of probucol, used as reference compounds. This effect is probably due to the synergistic action of hydroxytyrosol, oleuropein aglycones, and some flavonoids, such as quercetin, luteolin, and apigenin present in the virgin olive oil extract in minute amounts. In addition, changes in electrophoretic mobility of apo B are also prevented by the phenols.

Pure hydroxytyrosol (HT) and oleuropein (OE) both potently and dose-dependently inhibit copper sulfate-induced oxidation of LDL at concentrations of 10^{-6} to 10^{-4} M [7,8]. The free radical scavenging activities of hydroxytyrosol and oleuropein have been further confirmed [8,9] by the use of metal-independent oxidative systems and stable free radicals, such as DPPH [10a], in a series of experiments that demonstrated both a strong metal-chelation and a free-radical scavenging action. As far as the mechanism of action of olive oil phenolics is concerned, it is well-known that the antioxidant properties of *o*-diphenols are related to hydrogen-donation, which is their ability to improve radical stability by forming an intramolecular hydrogen bond between the free hydrogens of their hydroxyl group and their phenoxyl radicals [10b]. Although specific investigations of olive oil phenols are yet to be carried out, studies performed on the structure-activity relationship of flavonoids indicated that the degree of antioxidant activity is strictly related to the number of hydroxyl substitutions [11].

The mutagenic properties of oxidatively-damaged DNA suggest that antioxidants might have protective activity toward tumor formation. Low concentrations of hydroxytyrosol (50 μM) are able to scavenge peroxynitrite and therefore prevent $ONOO^-$-dependent DNA damage and tyrosine nitration [12,13]; also, in a model of copper-induced DNA damage, the prooxidant activities of hydroxytyrosol (due to its copper-reducing properties) become evident at non-physiological concentrations (>500 μM) and are 40-fold weaker than those of the widely-employed reducing agent ascorbate [13]. Interestingly, two human intervention studies [14,15] confirmed these data *in vivo*, indicating that extra virgin olive oil might decrease DNA damage, hence lessening cancer risk.

Oleuropein increases the functional activity of immune-competent cells (macrophages), as demonstrated by a significant increase (58.7 ± 4.6%) in the lipopolysaccharide (LPS)-induced production of nitric oxide, a bactericidal and cytostatic agent [10a]. This increase is consequent to a direct tonic effect of oleuropein on the inducible form of the enzyme nitric oxide synthase (iNOS), as demonstrated by Western blot analysis of cell homogenates and by coincubation of LPS-challenged cells with the iNOS inhibitor L-nitromethylarginine methylester [10b].

A correlation between inflammation and cardiovascular diseases has long been established; monocyte/macrophages and NF-κB play a pivotal role. The effects of an extra-virgin olive oil extract, particularly rich in phenolic compounds, were investigated on NF-κB translocation in monocytes and monocyte-derived macrophages (MDM) isolated from healthy volunteers. In a concentration-dependent manner, the extra-virgin olive oil extract inhibited p50 and p65 NF-kB translocation in both unstimulated and phorbol-myristate acetate (PMA)-challenged cells, being particularly effective on the p50 subunit. Interestingly, this effect occurred at concentrations found in human plasma after nutritional ingestion of virgin olive oil and was quantitatively similar to that exerted by ciglitazone, a PPAR-γ ligand. However, the extra-virgin olive oil extract did not affect PPAR-γ expression in monocytes and MDM. These data provide further evidence of the beneficial effects of extra-virgin olive oil by indicating its ability to inhibit NF-κB activation in human monocyte/macrophages [16].

***In vivo* studies:** Experimental evidence that phenolic compounds of different origin are absorbed from the diet is accumulating. Animal studies in rats and rabbits demonstrated that LDL isolated from animals fed virgin olive oil exhibit a higher resistance to oxidation when compared with animals given a triglyceride preparation with an equivalent amount of oleic acid, i.e. triolein [17], or «plain» olive oil [18]. We demonstrated that olive oil phenolics are dose-dependently absorbed in humans and that they are excreted in the urine, mainly as glucuronide

conjugates; it is noteworthy that increasing amounts of phenolics administered with olive oil stimulated the rate of conjugation with glucuronide [18]. These data add to the growing experimental evidence that indicates absorption and urinary disposition of flavonoids in humans [19].

It is noteworthy that HT exists in the brain as an endogenous catabolite of catecholic neurotransmitters, such as dopamine and norepinephrine [20], but its presence in urine has never, until recently, been described. On the other hand, the formation of homovanillic alcohol (HVAlc), the *O*-methylated derivative of HT, was reported by Manna et al [21] in human Caco-2 cell incubated with HT. We also reported the urinary excretion of HVAlc, in large excess over its basal excretion (57 ± 3 µg excreted in 24 hours, means ± SD, n= 6). We also described the substrate-induced enhancement of HVA formation, also a product of catecholamines metabolism, in addition to its basal urinary excretion (1660 ± 350 µg excreted in 24 hours, means ± SD, n= 6). Indeed, the results reported suggest that HT increases the basal excretion of HVA, even at the low doses of phenols administered. Future investigations will adopt commercially available virgin olive oils, thus allowing the further elucidation of the *in vivo* kinetics of olive oil phenolics in habitual consumption quantities.

In terms of biological activities, Covas *et al*. recently reviewed approximately 15 human intervention studies, the vast majority of which indicate that extra virgin olive oil (rich in phenols) is superior to seed oils and olive oil devoid of phenols in modulating selected surrogate markers of cardiovascular disease [22]. One example is an investigation of the effects of olive oil phenols on post prandial events. Bogani *et al*. evaluated the effects of moderate, real life doses of two olive oils, differing only in their phenolic content, on some *in vivo* indexes of oxidative stress (plasma antioxidant capacity and urinary hydrogen peroxide levels) in a post prandial setting. Moreover, the authors assessed whether phenolic compounds influence a few arachidonic acid metabolites involved in the atherosclerotic processes, such as leukotriene B_4 (LTB_4) and thromboxane B_2 (TXB_2). Six subjects in each group received the three oils [30 mL/day of olive oil (OO), corn oil (CO), or extra virgin olive oil (EVOO), distributed among meals] in a Latin square design. The results demonstrate that EVOO is capable of reducing the post prandial events that associate with inflammation and oxidative stress [23].

The effect of EVOO on platelet aggregation and plasma concentrations of homocysteine (Hcy) redox forms, in relation to the phenolic compounds' concentration, was also investigated in rats. Three olive oil samples with similar fatty acid, but different phenolic compound concentrations were used: refined olive oil (RF) with traces of phenolic compounds (control oil), native extra virgin olive oil with low phenolic compounds concentration (LC), and extra virgin olive oil with high phenolic compounds concentration (HC) enriching LC with its own phenolic compounds. Oil samples were administered to rats by gavage (1.25 mL/kg body weight) using two experimental designs: acute (24 h food deprivation and killed 1 h after oil administration) and subacute (12 d treatment, a daily dose of oil for 12 d, and killed after 24 h of food deprivation).

Platelet aggregation was induced by ADP (*ex vivo* tests) and a reduction in platelet reactivity occurred in cells from rats given LC in the subacute study and in cells from rats administered HC in both studies, as indicated by an increase in the agonist half maximal effective concentration. HC inhibited platelet aggregation induced by low ADP doses (reversible aggregation) in cells of rats in both the acute and subacute studies, whereas LC had this effect only in the subacute experiment. Moreover, in rats administered HC in both experiments, the plasma concentration of free reduced Hcy (rHcy) was lower and Hcy bound to protein by disulfide bonds (bHcy) was greater than in RF-treated rats. bHcy was also greater in rats given LC than in RF-treated rats in the subacute experiment. Plasma free-oxidized Hcy was greater in rats given LC and HC than in those administered RF only in the subacute experiment. These results show that phenolic compounds in EVOO inhibit platelet aggregation and reduce the plasma rHcy concentration, effects that may be associated with cardiovascular protection [24].

In conclusion, the biologically relevant properties of olive phenolics described in this article, although still to be further investigated in other controlled clinical trials, provide evidence to support the hypothesis that virgin olive oil consumption may contribute to lower CHD mortality.

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