Components of Olive Oil and Chemoprevention of Colorectal Cancer

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Olive oil contains a vast range of substances such as monounsaturated free fatty acids (e.g., oleic acid), hydrocarbon squalene, tocopherols, aroma components, and phenolic compounds. Higher consumption of olive oil is considered the hallmark of the traditional Mediterranean diet, which has been associated with low incidence and prevalence of cancer, including colorectal cancer. The anticancer properties of olive oil have been attributed to its high levels of monounsaturated fatty acids, squalene, tocopherols, and phenolic compounds. Nevertheless, there is a growing interest in studying the role of olive oil phenolics in carcinogenesis. This review aims to provide an overview of the relationship between olive oil phenolics and colorectal cancer, in particular summarizing the epidemiologic, in vitro, cellular, and animal studies on antioxidant and anticarcinogenic effects of olive oil phenolics.

Key words: olive oil phenolics, colorectal cancer, antioxidant, anticancer

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INTRODUCTION

The relationship between diet and cancer revolves around the issue of imbalances—either inadequacies or excesses of nutrients.1 Currently, a wealth of studies on the Mediterranean-style diet identifies it as a balanced diet with great potential to prevent the onset of diseases associated with oxidative damage (such as cardiovascular problems, aging, and cancer).2-7 Olive oil is a key component in the Mediterranean diet.2,6 The ability of olive oil and its phytochemicals to modulate the risks of such diseases has been extensively studied in various animal model, human intervention, and in vitro studies.8-14 Of particular interest is colorectal cancer, which is the fourth most common cause of cancer-related mortality in the world.15 Despite the growing body of knowledge pertaining to the effects of olive oil and its phytochemicals against colorectal cancer, the interpretation of the inverse relationship between the two subjects as causal is still being questioned. Thus, elucidating the molecular mechanisms by which olive oil and its components impart their protective effects is crucial.

THE COMPONENTS OF THE MEDITERRANEAN DIET

The Mediterranean diet is closely tied to areas of olive oil cultivation in the Mediterranean region in the late 1950s and early 1960s, before the invasion of the fast food culture in that area.6 The diet has been studied extensively over the past several years because of the low incidence of chronic diseases and the high life-expectancy rates attributed to the populations who eat a traditional Mediterranean diet.16 The components of the traditional Mediterranean diet are: a high monounsaturated to saturated fat ratio; moderate alcohol consumption; a high consumption of legumes, cereals, fruits, and vegetables; low consumption of meat and meat products; and moderate consumption of milk and dairy products.6

The total fat consumption in the Mediterranean population is high: between 30% and 40% of total caloric intake.16 Olive oil, which has been recognized as a major food group in the Mediterranean diet pyramid, explains the high ratio of monounsaturated to saturated fat.6

With respect to the relationship between the Mediterranean diet and colorectal cancer, it has been estimated that the incidence of the cancer could be reduced by 25% if the populations of developed Western countries would consume a traditional Mediterranean diet.6 Although there are no appreciable associations of the effects of individual components on colorectal cancer compared with the intake of the whole diet,17 the role of
olive oil as the key factor in the reduction of colorectal cancer risk is undeniable.

COLORECTAL CANCER

Colon cancer affects men and women almost equally, with a worldwide incidence of 9.4% in men and 10.1% in women, but cancer of the rectum is more common in men than in women, with a 20% to 50% higher incidence. However, population incidence rates for colorectal cancer vary 20-fold throughout the world, which is usually attributed to environmental and dietary factors. In particular, Mediterranean countries have lower rates of colorectal cancer compared with other Western countries. For example, colorectal cancer mortality in Greece is about 40% lower than that in the United Kingdom. Certain epidemiologic associations are consistently strong: diets high in meat, fat, and protein and low in vegetables and cereals are associated with increased colorectal cancer risk. Increased risk is also associated with higher alcohol consumption, while higher physical activity reduces the risk of colon cancer but not rectal cancer.

Currently, strategies for combating cancer include surgery, radiation, immunotherapy, gene therapy, chemotherapy and chemoprevention, screening and adenoma removal, as well as dietary modification and prevention. With all of the current interest in functional foods and so-called “nutraceuticals,” efforts have now been directed toward the use of such products for chemoprevention. In light of this, olive oil has been recognized as one of the plant sources with chemoprotective properties against colorectal cancer because of its ability to modulate various pathways in the colorectal cancer pathogenesis.

OLIVE OIL AND ITS PHYTOCHEMICALS

The olive tree, *Olea europea*, has rather strict climatic requirements for successful growth and fruit production, hence its extensive cultivation in the Mediterranean basin. The olive fruit is a drupe, oval in shape and consisting of two main parts: the pericarp and the endocarp (the pit or kernel, which contains the seeds). The pericarp is composed of the epicarp (skin) and the mesocarp (pulp). The pericarp contains 96% to 98% of the total amount of oil, with the remaining 2% to 4% in the kernel.

Edible olive oil constituents can be divided into saponifiable (98.5% to 99.5% of the oil) and unsaponifiable (0.5% to 1.5% of the oil) fractions. The saponifiable fraction includes fatty acids and triacylglycerols; the unsaponifiable fractions include hydrocarbons (squalene and carotenoids), chlorophylls, tocopherols, aliphatic alcohols, sterols, phenolic compounds, and volatile compounds.

Comprehensive data on quality and standard specifications, factors affecting quality, and adulteration of olive oil are provided by the Codex Alimentarius Commission, the Commission of the European Union, and the International Olive Oil Council. Kiritsakis and Markakis have previously provided a comprehensive review on olive oil and its related issues. The International Olive Oil Council has defined the classifications of olive oil as the following: virgin olive oil, refined olive oil, and olive oil-pomace oil. Virgin olive oil is the oil obtained from the fruit of the olive tree solely by mechanical or physical means (the cold-pressed technique), which do not lead to alterations of the oil. Virgin olive oil does not undergo any treatment other than washing, decantation, centrifugation, and filtration. Based on the acidity, virgin olive oil is further classified into extra virgin olive oil, virgin olive oil, and ordinary virgin olive oil (maximum free acidity: 1.0 g/100 g, 2.0 g/100 g, and 3.3 g/100 g, respectively). Virgin olive oil is regarded as a unique dietary lipid compared with other classes of olive oil in the sense that it retains the compounds that the fruit develops in response to environmental stress, most of which have a phenolic structure, where they constitute the polar minor components fraction.

POTENTIAL BENEFICIAL EFFECTS OF OLIVE OIL COMPONENTS

An ecological study comprising 28 countries from four continents reported that 76% of the inter-country variation in colorectal cancer incidence rates were explained by three significant dietary factors—meat, fish, and olive oil—in combination. Meat and fish were positively associated, and olive oil was negatively associated. Three out of six case-control studies undertaken in Mediterranean populations showed a weak inverse association between monounsaturated to saturated lipid ratio intake and colorectal cancer. The overall epidemiologic evidence concerning the association of olive oil intake and colorectal cancer based on case-control studies, although promising, is quantitatively limited and qualitatively suboptimal. Nevertheless, there has been much interest in identifying which components of olive oil have the most potent effects on carcinogenesis. Attention has focused on fatty acids, vitamin E, squalene, and phenolics.

Fatty Acids

The health-promoting properties of olive oil have been until recently been exclusively attributed to its high
monounsaturated fatty acid (oleic acid) content.\textsuperscript{2,30,31} According to the Recommended International Standard for Olive Oil, Virgin and Refined, the fatty acid composition is determined as follows (percentage mol/mol): oleic acid (56.0–83.0), palmitic acid (7.5–20.0), linoleic acid (3.5–20.0), stearic acid (0.5–3.5), palmitoleic acid (0.3–0.5), linolenic acid (0.0–1.5), myristic acid (0.0–0.5), and other fatty acids in minute amounts.\textsuperscript{7,27} The ratio of olive oil’s monounsaturated (MUFA) to polyunsaturated (PUFA) to saturated (SFA) fatty acids is 75%: 9%: 13%.\textsuperscript{32}

An increase in fecal bile acids is postulated to induce progression from normal mucosa to adenoma and carcinoma.\textsuperscript{5} MUFAs yield lower levels of bile acids than do PUFAs.\textsuperscript{7} While there are various pieces of epidemiologic evidence supporting the relationship between dietary fat and colorectal cancer,\textsuperscript{7} this has recently been challenged. Cumulative evidence from recent research has concluded that fat intake is not associated with colorectal cancer,\textsuperscript{33} and there is little evidence for a differential effect by fat types.\textsuperscript{20} Any association of fat intake to colorectal cancer appears to be attributable to the association with red meat intake rather than the reverse.\textsuperscript{33} Studies in cell cultures\textsuperscript{10} and in animals\textsuperscript{8} on the effects of oleic acid from olive oil show chemopreventive activity against colorectal cancer, but both suggest that other minor constituents from olive oil (including the phenolic compounds) could also act either synergistically or in other pathways, leading to the chemopreventive effects.

### Other Components of Olive Oil

The uncertainties of the role of MUFAs in modulating the risk of colorectal cancer has led researchers to direct their attention to other constituents that may be unique to olive oil. It is proposed that the high squalene content of olive oil (424 ± 21 mg/kg\textsuperscript{13} or 0.2% to 0.7%) compared with other human foods is a major factor in the cancer risk-reducing effect of olive oil.\textsuperscript{34} Squalene may inhibit hydroxymethyl glutaryl-coenzyme A reductase activity in colonic mucosal cells, leading to the suppression of azoxymethane-induced colonic aberrant crypt foci. Alternatively, it is possible that dietary squalene could modulate the biosynthesis of bile acids induced in tumor promotion.\textsuperscript{34}

Olive oil is also a source of vitamin E,\textsuperscript{16} a well-accepted major antioxidant in lipid tissue, which may play a role in cancer defenses. A compilation of animal and epidemiologic evidence showing an inverse relationship between vitamin E and cancer has been reported by Kelloff et al.\textsuperscript{35} and Liang et al.\textsuperscript{36} However, other studies, such as a prospective study on the association between supplemental vitamin E and colon cancer in men and women\textsuperscript{37} and in young or old mice,\textsuperscript{38} do not provide substantial evidence for a link between vitamin E supplement use and colon cancer risk. The main homologue of vitamin E forms present in olive oil is alpha-tocopherol, which makes up approximately 95% of total tocopherols (the other 5% are beta and gamma-tocopherols).\textsuperscript{27} Total tocopherol content of virgin olive oil was reported to be approximately 200 mg/kg (0.2% wt/wt)\textsuperscript{39} or in the range of 98 to 370 mg/kg.\textsuperscript{40} A high level of PUFAs is required for vitamin E uptake in the body,\textsuperscript{36} and PUFA levels in olive oil are low.\textsuperscript{32}

As discussed above, it has been difficult to establish MUFAs, vitamin E, or squalene as modulators of colorectal cancer. However, the shortcomings in these findings have triggered research into other components, in particular phenolics.

### OLIVE OIL PHENOLICS AS CHEMOPREVENTIVE AGENTS AGAINST COLORECTAL CANCER

Phenolic compounds are believed to function in the defense mechanism of plant cells against injury during oxidation processes.\textsuperscript{58} The term “phenolics” and “polyphenols” have been interchangeably used in the literature to refer generically to the presence of this basic molecular structure regardless of the number of hydroxy groups. Nevertheless, Boskou\textsuperscript{27} commented that the phenolic compounds of olive oil have been incorrectly referred to as polyphenols, since not all of them are polyhydroxy derivatives.

Plant-derived phenolic compounds may inhibit carcinogenesis at the initiation, promotion, and progression stages.\textsuperscript{41} A popular belief is that dietary phenolics are anticarcinogens because they are antioxidants, but direct evidence for this supposition is lacking.\textsuperscript{42,43} There are several mechanistic considerations of phenolics as anticarcinogens, as reviewed by Yang et al.\textsuperscript{43} (Table 1).

#### Table 1. Anticarcinogenic Properties of Phenolic Compounds\textsuperscript{43}

- Inhibit carcinogen activation, commonly catalyzed by cytochrome P\textsubscript{450} enzymes
- Induce phase II enzymes, which then facilitate the elimination of certain carcinogens or their reactive metabolites
- Inhibit arachidonic acid metabolism, where metabolism of arachidonic acid leads to the production of pro-inflammatory or mitogenic metabolites
- Modulate oncogenes, tumor suppressor genes, and signal transduction pathways leading to the inhibition of cell proliferation, transformation, angiogenesis, and induction of apoptosis
- Quench or prevent the formation of reactive oxygen and nitrogen species
Olive oil is a source of at least 30 phenolic compounds. The concentrations and the relative proportions of olive oil phenolics depend on several factors, including the cultivars, the soil, the climate, the manner in which the oil is produced and stored, and the degree of drupe maturation. The total phenol content of olive oil has been reported in numerous studies (Table 2). However, there are inconsistencies in the concentrations obtained, probably due to different methods of analysis employed in the studies.

Montedoro et al., reported a wide range of phenols for Italian olive oils (50–1000 mg/kg). The most recent study reported that the value for total phenolics of virgin olive oil is 232 ± 15 mg/kg. The most abundant phenolic compound in olive drupes is oleuropein, a bitter glycoside that constitutes up to 14% of the fruit's dry weight. Upon maturation, oleuropein undergoes enzymatic and nonenzymatic hydrolysis and yields several simpler compounds that can be found in virgin olive oil. The phenolic compounds highest in concentration in olive oil are the lignans (+)-1-acetoxy-pinoresinol and (+)-pinoresinol, followed by simple phenols derived from oleuropein (hydroxytyrosol and tyrosol) and secoiridoids (oleuropein, the aglycone of ligstroside and their respective decarboxylated dialdehyde derivatives; Figure 1). Simple phenols, secoiridoids, and lignans in extra virgin olive oils represented 47% of total phenols, whereas approximately 50% of total phenols were not quantified individually.

Currently, there is a growing interest in studying phenolic compounds in olive mill waste water. Large volumes of water are generated during olive oil production, particularly during malaxation process (continuous washing of olive paste with warm water prior to the procedure of separation of the oil from the paste). According to their partition coefficients, some of the bioactive compounds such as water-soluble phenolics end up in this major by-product water phase called waste water or vegetable water. In Italy alone, approximately 800,000 tons of olive mill waste water are generated yearly and discarded due to a failure to develop suitable end-of-pipe treatment technology. Di Giovacchino et al. reported that higher concentrations of total phenolics and o-diphenols were formed in the waste water compared with the oily phase of olive oil production (Table 2), which suggests that they could be recovered for a cheap source of natural antioxidants.

The efficacy of bioactive food or plant components could in large part be influenced by the amount available in the body at the target tissue level to exert their physiological effect; in other words, their bioavailability. In the case of olive oil phenolic compounds, there are very limited studies on bioavailability. Studies in rats showed that oral bioavailability estimates of hydroxytyrosol when administered in an olive oil solution and when dosed at an aqueous solution were 99% and 75%, respectively, while oral bioavailability estimates of tyrosol when orally administered in an olive oil and when dosed in an aqueous solution were 98% and 71%, respectively. Kinetic studies of hydroxytyrosol transport in Caco-2 monolayers demonstrate that it occurs by a passive diffusion mechanism with an apparent permeability coefficient similar to that of glucose, suggesting that absorption in the small intestine is 100%. The hydroxytyrosol and tyrosol are dose-dependently absorbed in humans and excreted in urine as glucuronide conjugates. Oleuropein is poorly absorbed from the isolated perfused rat intestine. The pharmacokinetic analysis of hydroxytyrosol indicates a fast and extensive uptake of the molecule by the organs and tissues investigated.

Interestingly, colon, being a major body site involved in the metabolism of phytochemicals, has direct contact with both metabolites and original dietary phenolics. Being fat soluble, the olive oil phenolics are likely to be absorbed and should have chemopreventive effects against breast cancer and other diseases such as heart disease. The unabsorbed phenolics will reach the large bowel, where they may have a chemopreventive effect against colorectal cancer. The amount of dietary phenols consumed per day probably exceeds 1 g, which supports the nutritional relevance of these compounds. In southern Italy, the dietary intake of olive oil can reach 50 g/d, corresponding to an average of about 25 mg of total phenols.

Even though hydroxytyrosol concentrations achievable in vivo by dietary intake of olive oil have not yet been evaluated, hydroxytyrosol in concentrations of up to 3 mM has been detected in oils with high total phenol content, far exceeding the level required for biological effects to occur. Excessive amounts of dietary phenols can be toxic, however, hydroxytyrosol did not show appreciable toxicity up to 2 g/kg body weight when orally administered to rats. Furthermore, cytotoxicity of phenolic compounds from olive oil was noted only at concentrations far exceeding those attainable after habitual consumption, thus indicating that the consumption of phenol-rich olive oil is safe.

Antioxidant Effects of Olive Oil

Antioxidant effects are perhaps the most studied properties of olive oil phenolics (Table 3). The antioxidant potential of olive oil phenolics was originally observed as a major factor in the high stability or shelf life of olive oil. The olive oil phenolics, acting as free radical scavengers, are chiefly responsible for the intrinsic defense against autoxidation of unsaturated fatty acids. The major contributor of this effect has been attributed to
<table>
<thead>
<tr>
<th>Sample/Cultivar</th>
<th>Type of Olive Oil/Method of Phenolic Compound Determination</th>
<th>Total Phenols</th>
<th>Hydroxytyrosol</th>
<th>Tyrosol</th>
<th>Secoiridoids</th>
<th>Lignan</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil on current Italian market</td>
<td>VOQ/HPLC (mean value)</td>
<td>232 ± 15</td>
<td>14.42 ± 3.01</td>
<td>27.45 ± 4.05</td>
<td>27.72 ± 6.84*</td>
<td>41.53 ± 3.93</td>
<td>Owen et al., 200011–13†</td>
</tr>
<tr>
<td></td>
<td>ROQ/HPLC (mean value)</td>
<td>62 ± 12</td>
<td>1.74 ± 0.84</td>
<td>2.98 ± 1.33</td>
<td>9.30 ± 3.81*</td>
<td>7.29 ± 2.56</td>
<td></td>
</tr>
<tr>
<td>Olive oil from Umbria, Apulia and Liguria, Italy</td>
<td>VOQ/Folin Ciocalteau assay (mg/kg of gallic acid)</td>
<td>50–1000</td>
<td>0–74.4</td>
<td>0.5–267.2</td>
<td>Determined as peak areas of HPLC chromatogram but not as mg/kg basis</td>
<td>ND</td>
<td>Montedoro et al., 199246,80‡</td>
</tr>
<tr>
<td>Olive oil from Picual and Hojiblanca cultivar (Spain)</td>
<td>VOQ/Folin Ciocalteau assay (mg/kg of caffeic acid)</td>
<td>150–350</td>
<td>Determined but data not shown</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Gutierrez et al., 199945§</td>
</tr>
<tr>
<td>Olive oil from various locations in Italy</td>
<td>VOQ</td>
<td>34.6 ± 1.8 to 114.3 ± 5.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Mosca et al., 200081‡</td>
</tr>
<tr>
<td></td>
<td>Folin Ciocalteau assay (mg/kg of caffeic acid)</td>
<td>47.5 ± 0.8 to 566.0 ± 6.2</td>
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<td></td>
<td>Spectrophotometric tyrosine-NADH enzymatic recycling assay</td>
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<tr>
<td>Olive oil from various regions in Greece</td>
<td>VOQ</td>
<td>2.0–69.6</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Tsimidou et al., 1992428</td>
</tr>
<tr>
<td></td>
<td>Folin Ciocalteau assay (mg/kg of caffeic acid)</td>
<td>18.7–242.51</td>
<td>0.7–39.4</td>
<td>0.8–29.8</td>
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<td></td>
<td>HPLC</td>
<td></td>
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<tr>
<td>Olive oil from various olive oil mills in Greece</td>
<td>VOQ</td>
<td>76–238</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Psomiadou et al., 2000408</td>
</tr>
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</table>
Table 2. (Cont’d) Phenolic Concentrations in Olive Oils and Olive Mill Waste Water

<table>
<thead>
<tr>
<th>Sample/Cultivar</th>
<th>Type of Olive Oil/Method of Phenolic Compound Determination</th>
<th>Total Phenols</th>
<th>Hydroxytyrosol</th>
<th>Tyrosol</th>
<th>Secoiridoids</th>
<th>Lignan</th>
<th>Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olive oil from Suri and Manzanillo varieties (Israel)</td>
<td>VOQ Folin Ciocalteau assay expressed as (mg/kg of caffeic acid): Chloroform/methanol extract</td>
<td>0.076–0.157</td>
<td>0.321–0.574</td>
<td></td>
<td></td>
<td></td>
<td>Gutfinger, 1981&lt;sup&gt;838&lt;/sup&gt;</td>
</tr>
<tr>
<td>Olive oils of good and poor quality (Italy)</td>
<td>VOQ</td>
<td>Method of extraction: Pressure Centrifugation</td>
<td>Expressed as mg/kg gallic acid: 96–292 74–158</td>
<td>Expressed as mg/kg caffeic acid: 38–263 25–237</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Good quality</td>
<td></td>
<td></td>
<td>74–158</td>
<td>25–237</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poor quality</td>
<td></td>
<td></td>
<td>61–135</td>
<td>55–86</td>
<td>77–118</td>
<td>59–70</td>
<td></td>
</tr>
<tr>
<td>Vegetable water Method of extraction:</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Good quality</td>
<td></td>
<td></td>
<td>4275–8865</td>
<td>3900–14,100</td>
<td>2227–5130</td>
<td>2200–6060</td>
<td></td>
</tr>
<tr>
<td>Poor quality</td>
<td></td>
<td></td>
<td>7659–9900</td>
<td>8880–13,560</td>
<td>2979–6593</td>
<td>2670–8160</td>
<td></td>
</tr>
<tr>
<td>Olives from different location in Italy, Spain and France</td>
<td>Olive mill waste water/HPLC</td>
<td>Expressed as g/100 g of dry matter: 8.11–22.78</td>
<td>Expressed as g/100 g of dry matter: 1.2–9.79</td>
<td>Expressed as g/100 g of dry matter: 0.45–4.72</td>
<td>Expressed as g/100 g of dry matter: 0.5 (oleuropein derivatives)</td>
<td>ND</td>
<td>Visioli et al., 1999&lt;sup&gt;48&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

NADH: reduced nicotinamide adenine dinucleotide; ROQ: refined virgin olive oil; VOQ: virgin olive oil; ND = not determined.

*Not significant.

<sup>1</sup>Phenolics extracted using absolute methanol.
<sup>2</sup>Phenolics extracted using methanol/water 80:20 (v/v).
<sup>3</sup>Phenolics extracted using oil-in-hexane solution with water/methanol 60:40 (v/v).
hydroxytyrosol (3,4-dihydroxyphenylethanol). In contrast, tyrosol (p-hydroxyphenylethanol) appears to make little or no contribution to the stability of olive oil.63 The antioxidant activity of these orthodiphenol or catecholic groups are related to hydrogen donation: their ability to improve radical stability by forming an intramolecular hydrogen bond between the hydrogens of their hydroxyl group and their phenoxy radicals.66

The participation of excessive reactive oxygen species (ROS), a situation referred to as oxidative stress,67 in the etiology of several diseases is now supported by a wealth of experimental data.68 Antioxidants are necessary to counteract this oxidative stress.39 In relation to this, dietary antioxidants may play important functions, and these activities may be transferred to the recipient organisms.63 With respect to antioxidants from olive oil phenolics, previous research has been primarily focused on effects on pathologies associated with cardiovascular disease and, to a lesser extent, cancer. This is probably due to the established role of oxidation in the etiology of atherosclerosis and the important function provided by antioxidants of low-density lipoproteins against free radical attack58 compared with the ambiguous role that oxidative damage plays in the etiology of cancer.42

Nevertheless, the antioxidant potentials of olive oil phenolics in relation to human diseases have been assessed in various in vitro systems, as will be reviewed here with particular emphasis on its effects towards cancer. The correlation between oxidative stress and cancer has been envisioned in the past primarily in relation to DNA damage. Recent data reporting that ROS themselves can act as pivotal molecules in signal transduction and in modulating gene expression more closely correlate oxidants, antioxidants, and cancer initiation, promotion, and progression (reviewed in Manna et al.61).

The antioxidant capacity of the olive oil phenolic extracts was significantly correlated with the total phenolic content of the oil, and many of the individual components showed a significant correlation with antioxidant activity, the strongest being the lignans, followed by (in decreasing order): SID-2 (dialdehydic form of oleuropein aglycone lacking the carboxymethyl group),

![Figure 1. Structures of the phenolic compounds and their precursors detected in olive oil. I = ligstroside; II = oleuropein glucoside; III = aglycone of ligstroside; IV = aglycone of oleuropein glucoside; V = dialdehydic form of ligstroside aglycone lacking a carboxymethyl group (SID-2); VI = dialdehydic form of oleuropein glucoside aglycone lacking a carboxymethyl group (SID-1); VII = tyrosol; VIII = hydroxytyrosol; IX = acetoxypinoresinol; X = (+)-pinoresinol.]({{image}}). From Owen et al., 2000.Used with permission.)
hydroxytyrosol, tyrosol, and SID-1 (dialdehydic form of ligstroside aglycone lacking the carboxymethyl group).\textsuperscript{13}

Visioli et al.\textsuperscript{14} investigated the scavenging actions of hydroxytyrosol and oleuropein, and found that both compounds reduced the free radical 2,2-diphenyl-1-picrylhydrazyl radical with very low EC\textsubscript{50} values. Both compounds, especially hydroxytyrosol, elicited a good concentration-dependent scavenging effect when tested using the same system. This also confirms the structural requirement (a catechol group) needed for optimal scavenging activity.\textsuperscript{69} While the 2,2-diphenyl-1-picrylhydrazyl radical assay is a homogenous system, oleuropein has been shown to have a better antioxidant activity in the membranous system than in homogenous solution in LP-LUV test as compared to hydroxytyrosol.\textsuperscript{69}

Hydroxytyrosol and oleuropein showed free radical scavenging activities in DPPH assay.\textsuperscript{14}

Oleuropein showed better antioxidant activity in the membranous system than in homogenous solution in LP-LUV test as compared to hydroxytyrosol.\textsuperscript{69}

Hydroxytyrosol promoted deoxyribose damage in deoxyribose assay and promoted DNA damage in bleomycin-Fe\textsuperscript{III} system.\textsuperscript{70}

Oleuropein exhibited high capabilities as free radical scavenger but showed no pro-oxidant activity in DMPD assay, Cu(II) reduction assay and copper chelating capacity assay.\textsuperscript{71}

BHT = butyl hydroxy toluene; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; DMPD = N,N\textsubscript{,}N\textsubscript{,}-dimethyl-p-phenylenediamine dihydrochloride; LP-LUV test = spectrophotometric determination of the accumulation of products (conjugated dienes) of peroxidation induced by water-soluble peroxyl radical generator 2,2\texttextsuperscript{'}azobis(2-amidinopropane)-hydrochloride (AAPH), of linoleic acid (LA) in mixed dipalmitoylphosphatidylycholine/linoleic acid (DPPC/LA) unilamelar vesicles (LUVs).
PUFAs are susceptible to free radical attack, which starts the chain of lipid peroxidation, leading to the formation of hydroxyperoxides, which in turn degrade to malonaldehyde.\(^\text{67}\)

Human erythrocytes were selected to further elucidate the antioxidant properties of hydroxytyrosol due to their specific role as oxygen carriers, which renders them particularly vulnerable to oxidative hazards. Furthermore, the defense mechanisms mediated by the induction of protein synthesis are not operative in erythrocytes, due to the lack of transcriptional and translational machinery.\(^\text{61}\)

Results from two studies showed that hydroxytyrosol is likely to prevent hydrogen peroxide-induced cytotoxicity by acting as a chain-breaking inhibitor of lipid peroxidation and chelating iron ions.\(^\text{59,61}\) On the other hand, results from the erythrocyte study suggest that the antioxidant activity of hydroxytyrosol is not due to the induction of a newly synthesized antioxidant protein, since the protein synthesis in erythrocytes is not operative.\(^\text{61,67}\)

Owen et al.\(^\text{11,12,13}\) also demonstrated that major olive oil phenolics exhibited strong antioxidant properties by using a fecal matrix assay system. Of interest is that total phenols inhibit hydroxyl radical attack on salicylic acid directly, and also indirectly via inhibition of xanthine oxidase activity. Because the simple phenols hydroxytyrosol and tyrosol have no effect on xanthine oxidase activity when tested alone, it can be concluded that their mode of action is via proton donation. In contrast, the secoiridoids appear to mediate their effects predominantly through inhibition of xanthine oxidase, whereas lignans have a dual action.\(^\text{13}\) The identification of lignans as major components of the phenolic fraction of olive oils by Owen et al.\(^\text{47}\) is of considerable interest because animal, cellular, and metabolic studies have shown that lignans possess important biological effects that may contribute to their potential role as chemopreventive agents.\(^\text{13}\)

The results from a study on the protective effects of total olive oil phenols against oxidative stress in human cell erythrocytes and Caco-2 cells\(^\text{72}\) agree with the results of studies discussed above. The protective effects observable in the cellular systems were compared with in vitro antioxidant properties measured using the ferric reducing/antioxidant power assay, and the reducing ability of olive oil phenolics strictly paralleled their ortho-phenolic content. The measure of the total antioxidant capacity is more representative than the protective effect of a single component, possibly due to interaction among different antioxidants present in the total olive oil phenolics.\(^\text{72}\)

In another study, hydroxytyrosol was shown to lower the levels of hydroxyperoxides, DNA damage, and mRNA levels of glutathione peroxidase in oxidative-stress sensitive human prostate cells.\(^\text{73}\) It has also been shown that hydroxytyrosol suppresses the peroxynitrite-dependent nitration of tyrosine, and in particular protects the cell from peroxynitrite-induced DNA damage.\(^\text{74}\)

### Other Effects of Olive Oil

Apart from the antioxidant capabilities that may prevent cancer by efficiently preventing the mutagenic activity caused by oxidative stress,\(^\text{72}\) olive oil phenolic compounds can also interfere with other steps in the carcinogenic process (Table 4). Moreover, a large majority of therapeutic strategies are based on the apoptotic activities of anticancer drugs.\(^\text{61}\)

Della Ragione et al.\(^\text{9}\) showed that hydroxytyrosol induced cell death in quiescent and differentiated HL60 cells, a cell line established from promyelocytic acute leukemia but not in two colorectal cell lines (HT29 and Caco-2). Since inflammatory processes are involved in all steps of cancer transformation, these findings suggest the capability of hydroxytyrosol to reduce the lymphocytic response by inhibiting proliferation and inducing apoptosis. This might be particularly important at the intestinal level and in the prevention of colon cancer.\(^\text{9}\) With respect to the molecular basis of the hydroxytyrosol apoptotic effect, it is hypothesized that the programmed cell death is activated through p53-independent pathways.\(^\text{61}\)

In a similar study, hydroxytyrosol was shown to inhibit proliferation of HL60 cells, HT29, and HT29 clone 19A. Hydroxytyrosol may exert its antiproliferative effect by directly interfering with the cell-cycle progression by inhibiting cyclin-dependent kinase or inducing cyclin-dependent kinase inhibitors. It may also block some other messengers involved in cell proliferation.\(^\text{75}\)

Hepatic microsomal cytochrome P450 is important as a catalyst in the metabolism of endogenous and xenobiotic substrates. Oleuropein, but not hydroxytyrosol, was found to be a potent mechanism-based inactivator of cytochrome 3A enzymes. It was postulated that inhibition by oleuropein is associated with its ester oxygen linkage or its non-terminal exocyclic alkene bond.\(^\text{76}\)

In regard to cancer progression or the metastases process, hydroxytyrosol is able to efficaciously inhibit 5- and 12-lipoxygenase activities.\(^\text{77,78}\) These lipoxygenase (LOX) enzymes form different metabolites within the arachidonic acid pathway that appear to enhance tumorigenesis. 5-LOX metabolizes arachidonic acid to form 5-S-hydroxy-6,8,11,14-eicosatetraenoic acid (5-S-HETE) and leukotriene B\(_4\), while 12-LOX metabolizes arachidonic acid to 12-S-hydroxy-5,8,10,14-eicosatetraenoic acid.
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noic acid (12-S-HETE). 5-S-HETE metabolic products may inhibit proliferation of tumor cells and induce apoptosis. 12-S-HETE has been shown to promote invasion and metastasis by up-regulating adhesion molecules, increasing the adhesion of tumor cells to matrix protein fibronectin and microvessel endothelium, enhancing cell migration during tumorigenesis, and promoting tumor spread through modulation of protein kinase C-α (reviewed in Shureiqi et al. 79).

CONCLUSION

Olive oil appears to be an example of a functional food with a range of constituents that may contribute to its overall chemopreventive/beneficial effects.2 In particular, olive oil phenolic compounds have the potential to inhibit various stages of carcinogenesis and therefore may modulate cancer risk. Currently, data on the molecular mechanisms of olive oil phenolic compounds toward the prevention of colorectal cancer are very scarce. Studies are needed to contribute to the development of this promising area of chemoprevention.

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