Special Article

November 2005: 374–386

Components of Olive Oil and Chemoprevention of Colorectal Cancer

Yumi Z.H-Y. Hashim, M Eng, Chris I.R. Gill, PhD, Hugh McGlynn, PhD, and Ian R. Rowland, PhD

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

doi: 10.1301/nr.2005.nov.374–386

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.

Please address all correspondence to: Dr. Ian Rowland, Northern Ireland Centre for Food and Health, University of Ulster (Coleraine), Cromore Road, Coleraine, Co. Londonderry, Northern Ireland, United Kingdom BT52 1SA; Phone: 28-70323039; Fax: 28-70323023; E-mail: i.rowland@ulster.ac.uk.

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,[15] but cancer of the rectum is more common in men than in women, with a 20% to 50% higher incidence.[18] However, population incidence rates for colorectal cancer vary 20-fold throughout the world,[19] which is usually attributed to environmental and dietary factors. In particular, Mediterranean countries have lower rates of colorectal cancer compared with other Western countries.[6,18,20] For example, colorectal cancer mortality in Greece is about 40% lower than that in the United Kingdom.[19] 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.[21]

Currently, strategies for combating cancer include surgery, radiation, immunotherapy, gene therapy, chemotherapy and chemoprevention,[22] screening and adenoma removal,[23] as well as dietary modification and prevention.[1,23,24,25] 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,[26] 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.[26]

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.[4]

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.[26,27] Kiritsakis and Markakis[28] 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, 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).[4] 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.[29]

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.[5] 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.[6] 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.[7] 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.[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.[7,27] The ratio of olive oil's monounsaturated (MUFA) to polyunsaturated (PUFA) to saturated (SFA) fatty acids is 75%: 9%: 13%.[32]

An increase in fecal bile acids is postulated to induce progression from normal mucosa to adenoma and carcinoma.[5] MUFAs yield lower levels of bile acids than do PUFAs.[7] While there are various pieces of epidemiologic evidence supporting the relationship between dietary fat and colorectal cancer, [7] this has recently been challenged. Cumulative evidence from recent research has concluded that fat intake is not associated with colorectal cancer,[33] and there is little evidence for a differential effect by fat types.[20] Any association of fat intake to colorectal cancer appears to be attributable to the association with red meat intake rather than the reverse.[33] Studies in cell cultures[10] and in animals[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[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.[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. [34]

Olive oil is also a source of vitamin E,[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.[35] and Liang et al.[36] However, other studies, such as a prospective study on the association between supplemental vitamin E and colon cancer in men and women[37] and in young or old mice,[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).[27] Total tocopherol content of virgin olive oil was reported to be approximately 200 mg/kg (0.2% wt/wt)[39] or in the range of 98 to 370 mg/kg.[40] A high level of PUFAs is required for vitamin E uptake in the body,[36] and PUFA levels in olive oil are low.[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 CHEMOPROTECTIVE AGENTS AGAINST COLORECTAL CANCER

Phenolic compounds are believed to function in the defense mechanism of plant cells against injury during oxidation processes.[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[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.[41] A popular belief is that dietary phenolics are anticarcinogens because they are antioxidants, but direct evidence for this supposition is lacking.[42,43] There are several mechanistic considerations of phenolics as anticarcinogens, as reviewed by Yang et al.[43] (Table 1).

Table 1. Anticarcinogenic Properties of Phenolic Compounds[43]

- Inhibit carcinogen activation, commonly catalyzed by cytochrome P_{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.[44] 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,[14] and the degree of drupe maturation.[45] 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.,[46] 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.[11,12,13] 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.[29] The phenolic compounds highest in concentration in olive oil are the lignans (+)-1-acetoxypinoresinol 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[11,12,13,47]; 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.[11]

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).[44] 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[48] or vegetable water.[49] 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.[48] Di Giovacchino et al. [49] 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.[50] In the case of olive oil phenolic compounds, there are very limited studies on bioavailability.[3] 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.[51] 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%.[52] The hydroxytyrosol and tyrosol are dose-dependently absorbed in humans and excreted in urine as glucuronide conjugates.[53,54] Oleuropein is poorly absorbed from the isolated perfused rat intestine.[55] The pharmacokinetic analysis of hydroxytyrosol indicates a fast and extensive uptake of the molecule by the organs and tissues investigated.[56]

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[57] and other diseases such as heart disease.[2,58] The unabsorbed phenolics will reach the large bowel, where they may have a chemopreventive effect against colorectal cancer.[12] The amount of dietary phenols consumed per day probably exceeds 1 g, which supports the nutritional relevance of these compounds.[59] 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.[60]

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.[61] Excessive amounts of dietary phenols can be toxic,[43] however, hydroxytyrosol did not show appreciable toxicity up to 2 g/kg body weight when orally administered to rats.[56] 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.[62]

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.[63,64] The olive oil phenolics, acting as free radical scavengers, are chiefly responsible for the intrinsic defense against autoxidation of unsaturated fatty acids.[65] The major contributor of this effect has been attributed to

Table 2. Phenolic Concentrations in Olive Oils and Olive Mill Waste Water

		Olive Oil Phenolics (mg/kg)					
Sample/Cultivar	**Type of Olive Oil/ Method of Phenolic Compound Determination**	**Total Phenols**	**Hydroxytyrosol**	**Tyrosol**	**Secoiridoids**	**Lignan**	**Study**
Olive oil on current Italian market	VOQ/HPLC (mean value) ROQ/HPLC (mean value)	232 ± 15 62 ± 12	14.42 ± 3.01 1.74 ± 0.84	27.45 ± 4.05 2.98 ± 1.33	27.72 ± 6.84* 9.30 ± 3.81*	41.53 ± 3.93 7.29 ± 2.56	Owen et al., 2000[11–13†]
Olive oil from Umbria, Apulia and Liguria, Italy	VOQ/Folin Ciocalteau assay (mg/kg of gallic acid)	50–1000	0–74.4	0.5–267.2	Determined as peak areas of HPLC chromatogram but not as mg/kg basis	ND	Montedoro et al., 1992[46,80‡]
Olive oil from Picual and Hojiblanca cultivar (Spain)	VOQ/Folin Ciocalteau assay (mg/kg of caffeic acid)	150–350	Determined but data not shown	ND	ND	ND	Gutierrez et al., 1999[45§]
Olive oil from various locations in Italy	VOQ Folin Ciocalteau assay (mg/kg of caffeic acid) Spectrophotometric tyrosine-NADH enzymatic recycling assay	 34.6 ± 1.8 to 114.3 ± 5.1 47.5 ± 0.8 to 566.0 ± 6.2	ND	ND	ND	ND	Mosca et al., 2000[81‡]
Olive oil from various regions in Greece	VOQ Folin Ciocalteau assay (mg/kg of caffeic acid) HPLC	 2.0–69.6 18.7–242.51	 ND 0.7–39.4	 ND 0.8–29.8	ND	ND	Tsimidou et al., 1992[82§]
Olive oil from various olive oil mills in Greece	VOQ Folin Ciocalteau assay (mg/kg of caffeic acid)	 76–238	 ND	 ND	 ND	 ND	Psomiadou et al., 2000[40§]

Table 2. (Cont'd) Phenolic Concentrations in Olive Oils and Olive Mill Waste Water

		Olive Oil Phenolics					
Sample/Cultivar	**Type of Olive Oil/Method of Phenolic Compound Determination**	**Total Phenols**	**Hydroxytyrosol**	**Tyrosol**	**Secoiridoids**	**Lignan**	**Study**
Olive oil from Suri and Manzanillo varieties (Israel)	VOQ	Folin Ciocalteau assay expressed as (mg/kg of caffeic acid):	Determination by molybdate expressed as (mg/kg of caffeic acid):	ND	ND	ND	Gutfinger, 1981[83§]
		0.076–0.157	0.005–0.015				
	Chloroform/methanol extract	0.321–0.574	0.062–0.194				
Olive oils of good and poor quality (Italy)	VOQ	Expressed as mg/kg gallic acid:	Expressed as mg/kg caffeic acid:				Di Giovacchino et al., 1994[49]
	Method of extraction:						
Good quality	Pressure	96–292	38–263	ND	ND	ND	
	Centrifugation	74–158	25–237				
Poor quality	Pressure	61–135	55–86				
	Centrifugation	77–118	59–70				
	Vegetable water	Expressed as mg/kg gallic acid:	Expressed as mg/kg caffeic acid:	ND	ND	ND	Di Giovacchino et al., 1994[49]
	Method of extraction:						
Good quality	Pressure	4275–8865	3900–14,100				
	Centrifugation	2227–5130	2200–6060				
Poor quality	Pressure	7659–9900	8880–13,560				
	Centrifugation	2979–6593	2670–8160				
Olives from different location in Italy, Spain and France	Olive mill waste water/HPLC	Expressed as g/100 g of dry matter: 8.11–22.78	Expressed as g/100 g of dry matter: 1.2–9.79	Expressed as g/100 g of dry matter: 0.45–4.72	Expressed as g/100 g of dry matter: 0.5 (oleuropein derivatives)	ND	Visioli et al., 1999[48]

NADH: reduced nicotinamide adenine dinucleotide; ROQ: refined virgin olive oil; VOQ: virgin olive oil; ND = not determined.
*Not significant.
†Phenolics extracted using absolute methanol.
‡Phenolics extracted using methanol/water 80:20 (v/v).
§Phenolics extracted using oil-in-hexane solution with water/methanol 60:40 (v/v).

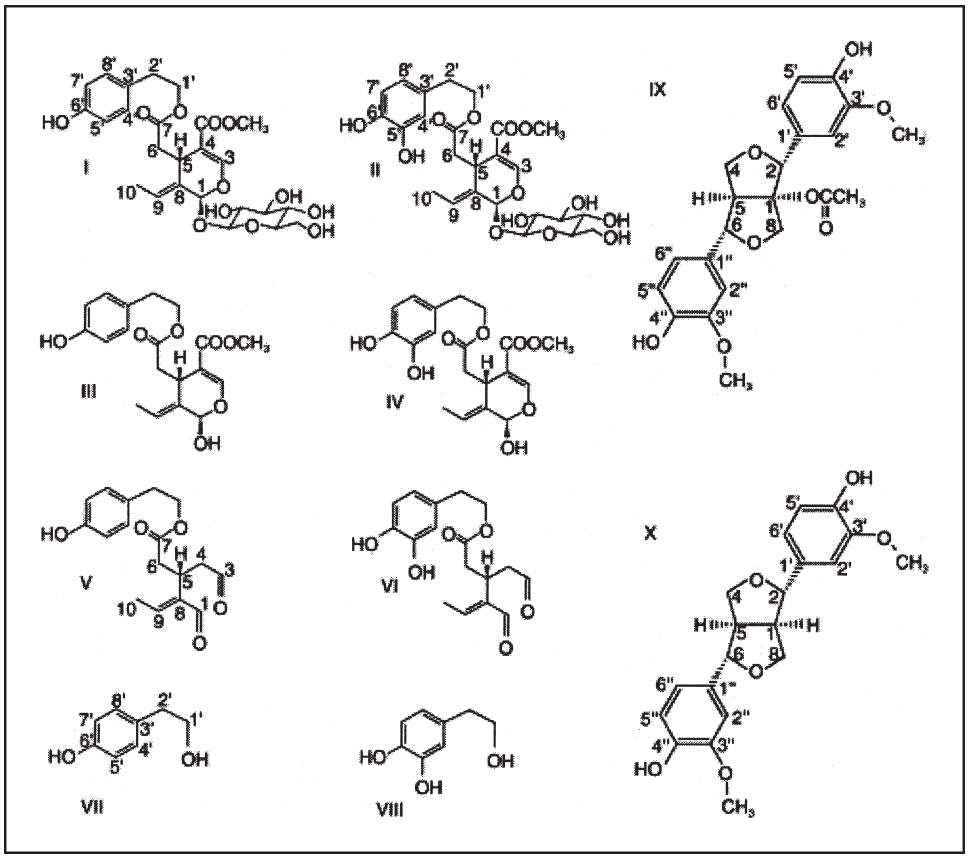

Figure 1. Structures of the phenolic compounds and their precursors detected in olive oil. I = ligstroside; II = oleuropein glucoside; III = aglycone of ligstroside; IV = aglycone of oleuropein glucoside; V = dialdehydic form of ligstroside aglycone lacking a carboxymethyl group (SID-2); VI = dialdehydic form of oleuropein glucoside aglycone lacking a carboxymethyl group (SID-1); VII = tyrosol; VIII = hydroxytyrosol; IX = acetoxypinoresinol; X = (+)-pinoresinol.[11] From Owen et al., 2000.[11] Used with permission.

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 phenoxyl 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 attack[58] 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),

Table 3. Studies of the Antioxidant Effects of Olive Oil Phenolic Compounds

Oil Rancidity/Shelf-Life Studies	
Hydroxytyrosol but not tyrosol showed protection factors greater than BHT in refined olive oil	Papadopoulos and Boskou, 1991[64]
Hydrophilic compounds (olive oil phenolics) but not lipophilic compounds (tocopherols) showed high correlation with oxidative stability of virgin olive oil	Baldioli et al., 1996[65]
In Vitro Studies	
Lignan showed strongest correlation with antioxidant activity of olive oil phenolics in hypoxanthine/xanthine oxidase system and faecal assay system	Owen et al., 2000[11,13]
Hydroxytyrosol and oleuropein showed free radical scavenging activities in DPPH assay	Visioli et al., 1998[14]
Oleuropein showed better antioxidant activity in the membranous system than in homogenous solution in LP-LUV test as compared to hydroxytyrosol	Saija et al., 1998[69]
Hydroxytyrosol promoted deoxyribose damage in deoxyribose assay and promoted DNA damage in bleomycin-FeIII system	Aesbach et al., 1994[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	Briante et al., 2003[71]

BHT = butyl hydroxy toluene; DPPH = 2,2-diphenyl-1-picrylhydrazyl radical; DMPD = *N*,*N*,-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′azobis(2-amidinopropane)-hydrochloride (AAPH), of linoleic acid (LA) in mixed dipalmitoylphosphatidylcholine/linoleic acid (DPPC/LA) unilamelar vesicles (LUVs).

hydroxytyrosol, tyrosol, and SID-1 (dialdehydic form of ligstroside aglycone lacking the carboxymethyl group).[13]

Visioli et al.[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_{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.[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, as exhibited in the LP-LUV test (oxidation of linoleic acid) system. Hydroxytyrosol is more hydrophilic than oleuropein and, unlike hydroxytyrosol, oleuropein seems to be located within biomembranes. It was hypothesized that hydroxytyrosol can serve as scavenger of aqueous peroxyl radicals near the membrane surface, while oleuropein acts also as a scavenger of chain-propagating lipid peroxyl radicals within the membrane.[69]

Hydroxytyrosol is amphiphilic. It could therefore provide useful protection against oxidative processes in emulsions or biological systems at the water-lipid interface.[70] However, its ability to cause pro-oxidant effects must be considered, as it had been shown to promote deoxyribose damage in the deoxyribose assay and promote DNA damage in the bleomycin-Fe III system.[70] Meanwhile, oleuropein was reported to behave as a powerful scavenger of free radicals, but does not possess pro-oxidant activity, as it does not chelate copper ion.[71]

Manna et al.[59, 61] clearly demonstrated that hydroxytyrosol at micromolar concentrations counteracts ROS-induced cytotoxicity in human cellular systems. Two systems were tested on Caco-2 human colonic cell lines: hydrogen peroxide and enzyme xanthine oxidase/xanthine system.[59] Among the cytotoxic effects of ROS on mammalian cells, particularly severe is the damage induced to membrane phospholipids. It is known that

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.[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.[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.[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.[61,67]

Owen et al.[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.[13] The identification of lignans as major components of the phenolic fraction of olive oils by Owen et al.[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.[13]

The results from a study on the protective effects of total olive oil phenolics against oxidative stress in human cell erythrocytes and Caco-2 cells[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 orthodiphenolic 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.[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.[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.[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,[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.[61]

Della Ragione et al.[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.[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.[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.[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.[76]

In regard to cancer progression or the metastases process, hydroxytyrosol is able to efficaciously inhibit 5- and 12-lipoxygenase activities.[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 leukotrine B_4, while 12-LOX metabolizes arachidonic acid to 12-S-hydroxy-5,8,10,14-eicosatetrae-

Table 4. Cellular Studies on Biological/Anticarcinogenic Effects of Olive Oil Phenolic Compounds

Effects/Outcome	Cell System	Concentration Range Tested (μM)	Effective Concentrations (μM)	Reference
Antioxidant effects				
Hydroxytyrosol counteracts ROS-induced cytotoxicity in H_2O_2 and xanthine oxidase/xanthine system	Human colonic cells (Caco-2)	H_2O_2 system: 100–250 Xanthine oxidase/ oxidase system: 50–100	Significant minimum inhibitory concentration H_2O_2 system: 250 μM; Xanthine oxidase/oxidase system: 100 μM	Manna et al., 1997[59]
Hydroxytyrosol prevents oxidative hemolysis and lipid peroxidation in H_2O_2 oxidation alteration system	Human erythrocytes from blood plasma	0–200	50 μM significant protection; 100 μM complete prevention from oxidative damage and lipid peroxidation	Manna et al., 1999[67]
Hydroxytyrosol lower the levels of hydroxyperoxides, DNA damage and mRNA levels of classic glutathine peroxidase (GPx)	Human prostate cells (PC3)	10–250	Minimum effective concentration: 10 μM	Quiles et al., 2002[73]
Hydroxytyrosol suppresses the peroxynitrite-dependent tyrosine nitration and DNA damage	Neuronal hybridoma N-18-RE-105 (primary rat embryonic retinal cell $\times$ mouse neuroblastoma cell)	0–1000	100 μM provides over 50% of inhibition of tyrosine nitration	Deiana et al., 1999[74]
Antiproliferative/apoptotic effect				
Hydroxytyrosol showed antiproliferative activity and induced apoptosis	White blood cell from promyelocytic leukemia (HL60)	0–200	50–100 μM	Della Ragione et al., 2000[9]
Hydroxytyrosol inhibit proliferation by interfering with the cell cycle progression (inhibit cyclin-dependent kinase, Cdk or invading Cdk inhibitors)	HL60 and human colon adenocarcinoma (HT29 and HT29 clone A)	0–250	EC_{50} for HL60 = 50 μM EC_{50} for HT29 = 750 μM	Fabiani et al., 2002[75]
Antimetastatic effect				
Hydroxytyrosol inhibits platelet 12-lipoxygenase and PMNL 5-lipoxygenase	Rat platelets from Wistar-King rats Rat peritoneal PMNL	0.01–100	IC_{50} = 4.2 μM IC_{50} = 13.0 μM	Kohyama et al., 1997[77]
Hydroxytyrosol inhibits 5-lipoxygenase activities (inhibit arachidonic acid metabolism)	Rat peritoneal polymorphonuclear leucocytes (PMNL)	40–200	EC_{50} = 15.0 μM	de la Puerta, 1999[78]

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.

REFERENCES

1. Williams AM, Wynder EL. Diet and cancer: a synopsis of causes and prevention strategies. In: Watson R, Mufti SI, eds. *Nutrition and Cancer Prevention.* Boca Raton, FL: CRC Press;1996; 1–12.
2. Stark AH, Madar Z. Olive oil as a functional food: epidemiology and nutritional approaches. Nutr Rev. 2002;60:170–176.
3. Tuck KL, Hayball P. Major phenolic compounds in olive oil: metabolism and health effects. J Nutr Biochem. 2002;13:636–644.
4. Alarcon de la Lastra, C, Barranco MD, Motilva V, et al. Mediterranean diet and health: biological importance of olive oil. Curr Pharm Des. 2001;7:933–950
5. Stoneham M, Goldacre M, Seagroatt V, et al. Olive oil, diet and colorectal cancer: an ecological study and a hypothesis. J Epidemiol Comm Health. 2000; 54:756–760.
6. Trichopolou A, Lagiou, P, Kuper H, et al. Cancer and Mediterranean dietary traditions. Cancer Epidemiol Biomarkers Prev. 2000;9:869–873.
7. Lipworth L, Martines ME, Angell J, et al. Olive oil and human cancer: an assessment of the evidence. Prev Med. 1997;26:181–190.
8. Bartoli R, Fernandez–Banares F, Navarro E, et al. Effect of olive oil on early and late events of colon carcinogenesis in rats: modulation of arachidonic acid metabolism and local prostaglandin E2 synthesis. Gut. 2000;46:191–199.
9. Della Ragione F, Cucciolla V, Borriello A, et al. Hydroxytyrosol, a natural molecule occurring in olive oil, induces cytochrome c dependent apoptosis. Biochem Biophys Res Comm. 2000;278:733–739.
10. Llor X, Pons E, Roca A, et al. The effects of fish oil, olive oil, oleic acid and linoleic acid on colorectal neoplastic processes. Clin Nutr. 2003;22:71–79.
11. Owen RW, Giacosa A, Hull WE, et al. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. Eur J Cancer. 2000;36:1235–1247.
12. Owen RW, Giacosa A, Hull WE, et al. Olive oil consumption and health: the possible role of antioxidants. Lancet Oncol. 2000;1:107–112.
13. Owen RW, Mier W, Giacosa A, et al. Phenolic compounds and squalene in olive oils:the concentration and antioxidants potential of total phenols, simple phenols, secoiridoids, lignans and squalene. Food Chem Toxicol. 2000;38:647–659.
14. Visioli F, Bellomo G, Galli C. Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Comm. 1998;247:60–64.
15. Boyle P, Langman JS. ABC of colorectal cancer. Epidemiology. Brit Med J. 2000;321:805.
16. Willett WC, Sacks F, Trichopoulou A, et al. Mediterranean diet pyramid:a cultural model for healthy eating. Am J Clin Nutr. 1995;61(suppl):1402S–1406S.
17. Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med. 2003;348:2599–2608.
18. World Cancer Research Fund (WCRF) and American Institute for Cancer Research (AICR). *Food, Nutrition, and the Prevention of Cancer: A Global Perspective.* Washington DC: AICR; 1997.
19. Ferlay J, Bray F, Pisani P, et al. *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide.* Lyon: IARC Press; 2004.
20. Braga C, La Vecchia C, Franceshi S, et al. Olive oil, other seasoning fats and the risk of colorectal carcinoma. Cancer. 1999;82:448–453.
21. Potter JD. Epidemiologic, environmental and lifestyle issues in colorectal cancer. In: Young GP, Rozen P, Levin B, eds. *Prevention and Early Detection of Colorectal Cancer.* London: WB Saunders; 1996; 23–44.
22. Karp G. Cancer. In: *Cell and Molecular Biology: Concepts and Experiments.* 3rd ed. New York: John Wiley & Sons; 2002.
23. Bertagnolli MM, McDougall CJ, Newmark HL. Colon cancer prevention:intervening in a multistage process. Proc Soc Exp Biol Med. 1997;216:266–274.
24. Schatzkin A. Dietary prevention of colorectal cancer. In: Young GP, Rozen P, Levin B, eds. *Prevention and Early Detection of Colorectal Cancer.* London: WB Saunders; 1996; 103–114.
25. American Cancer Society. *Overview: Colon and Rectum Cancer.* Available online at: http://www.cancer.org. Accessed October 24, 2005.
26. Kiritsakis AK. Olive fruit – olive oil formation. In Kiritsakis AK, ed. *Olive Oil.* Champaign, IL: American Oil Chemistry Society; 1991; 12–25.
27. Boskou D. Olive oil. World Rev Nutr Diet. 2000;87: 56–77.
28. Kiritksakis A and Markakis P. Olive oil: a review. In: *Advances in Food Research.* London: Academic Press; 1987.
29. Visioli F, Galli C. Olive oil:more than just oleic acid. Am J Clin Nutr 2000;72:853
30. Serra-Majem LI, Ngo de la Cruz J, Ribas L, et al, Olive oil and the Mediterranean diet: beyond the rhetoric. Eur J Clin Nutr. 2003;57(suppl 1):S2–S7.

31. Visioli F, Galli C. Biological properties of olive oil phytochemicals. Crit Rev Food Sci Nutr. 2002;42: 209–221.
32. Siew WL, Cheah KY, Idris NA. Novel oil compositions. Malaysian Palm Oil Board Information Series. 2003;194:208.
33. Kushi L, Giovanucci E. Dietary fat and cancer. Am J Med. 2002;113:9B.
34. Rao CV, Newmark HL, Reddy BS. Chemopreventive effecet of squalene on colon cancer. Carcinogenesis. 1998;19:287–290.
35. Kelloff GJ, Boone CW, Sigman CC, et al. Chemoprevention of colorectal cancer. In: Young GP, Rozen P, Levin B, eds. *Prevention and Early Detection of Colorectal Cancer.* London: WB Saunders; 1996; 115–140.
36. Liang B, Wang JW, Watson RR. Vitamin E and cancer prevention. In: Watson RR, Mufti SI, eds. *Nutrition and Cancer Prevention.* Boca Raton, FL: CRC Press; 1995; 283–298.
37. Chung H, Wu D, Han SN, et al. Vitamin E supplementation does not alter azoxymethane-induced colonic aberrant crypt foci formation in young or old mice. J Nutr. 2003;133:528–532.
38. Wu K, Willett WC, Chan JM, et al. A prospective study of supplemental vitamin E intake and risk of colon cancer in women and men. Cancer Epidemiol Biomarkers Prev. 2002;11:1298–1304.
39. Visioli F. Antioxidants in Mediterranean diet. World Rev Nutr Diet. 2000;87:43–55.
40. Psomiadou E, Tsimidou M, Boskou D. Alpha-tocopherol content of Greek virgin olive oils. J Agric Food Chem. 2000;48:1770–1775.
41. Mahmoud NN, Carothers AM, Grunberger D, et al. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis. 2000;21:921–927.
42. Lund E. Olive oil phenolics:effects on DNA-oxidation and redox enzyme mRNA in prostate cells. Brit J Nutr. 2002;88:223–224.
43. Yang CS, Landau JM, Huang MT, et al. Inhibition of carcinogenesis by dietary polyphenolic compounds. Ann Rev Nutr. 2001;21:381–406.
44. Boskou D. *Olive Oil Chemistry and Technology.* Champaign, IL: AOCS Press; 1996.
45. Gutierrez F, Jimenez B, Ruiz A et al. Stability of virgin olive oil extracted from the varieties picual and hojiblanca and the different components involved. J Agric Food Chem. 1999;47:121–127.
46. Montedoro G, Servili M, Baldioli M, et al. Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. J Agric Food Chem. 1992;40:1577–1580.
47. Owen RW, Mier W, Giacosa A, et al. Identification of lignans as major components in the phenolic fraction of olive oil. Clin Chem. 2000;46:976–988.
48. Visioli F, Romani A, Mulinacci N, et al. Antioxidant and other biological activities of olive mill waste waters. J Agric Food Chem. 1999;47:3397–3401.
49. Di Giovacchino L, Solinas M, Miccoli M. Effect of extraction systems on the quality of virgin olive oil. JAOCS. 1994;71:1189–1194.
50. Picciano MF. Bioavailability: a key factor in the efficacy of bioactive food components. Nutr Today. 2002;37:206–208.
51. Tuck KL, Freeman MP, Hayball PJ, et al. The in vivo fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds of rats. J Nutr. 2001;131:1993–1996.
52. Manna C, Galletti P, Maisto G, et al. Transport mechanism and metabolism of olive oil hydroxytyrosol in Caco-2 cells. FEBS Lett. 2000;470:341–344.
53. Caruso D, Visioli F, Patelli R, et al. Urinary excretion of olive oil phenols and their metabolites in humans. Metab Clin Exp. 2001,50:1426–1428.
54. Visioli F, Galli C, Bornet F, et al. Olive oil phenolics are dose-dependently absorbed in humans. FEBS Lett. 2000;468:159–160.
55. Edgecombe SC, Stretch GL, Hayball PJ. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. J Nutr. 2000;13:2996–3002.
56. D'Angelo S, Manna C, Migliardi V. et al. Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. Drug Metab Disposition. 2001;29:1492–1498.
57. Simonsen NR, Najavas, JF-C, Martin-Moreno JM, et al. Tissue stores of individual monounsaturated fatty acids and breast cancer: the EURAMIC study. Am J Clin Nutr. 1998;68:134–141.
58. Trichopoulou A, Vasipoulou E, Lagiou, A. Mediterranean diet and coronary heart disease:are antioxidants critical? Nutr Rev. 1999;57:253.
59. Manna C, Galletti P, Cucciolla V, et al. The protective effect of the olive oil polyphenols (3,4-dihydroxyphenyl-ethanol) counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. J Nutr. 1997;127:286–292.
60. Ferro-Luzzi A, Sette S. The Mediterranean diet:an attempt to define its present and past composition. Eur J Clin Nutr. 1989;43(suppl 2):13–29.
61. Manna C, Della Ragione F, Cucciolla, V, et al. Biological effects of hydroxytyrosol, a polyphenol from olive oil endowed with antioxidant activity. In: Zappia V, ed. *Advances in Nutrition and Cancer.* New York: Kluwer Academic/Plenum Publishers; 1999.
62. Babich H, Visioli F. In vitro cytotoxicity to human cells in culture of some phenolics from olive oil. Il Farmaco. 2003;58:403–407.
63. Galli C, Visioli F. Antioxidant and other activities of phenolics in olives/olive oil, typical components of the Mediterranean diet. Lipids. 1999;34(suppl):S23–S26.
64. Papadopoulos G, Boskou D. Antioxidant effect of natural phenols on olive oil. Journal of the American Oil Chemists' Society. 1991;68(9)669–671.
65. Baldioli M, Servilli M, Pevretti G, et al. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J Am Oil Chem Soc. 1996;73:1589–1593.
66. Visioli F, Galli C. Olive oil phenols and their potential effects on human health. J Agric Food Chem. 1998; 46:4292–4296.
67. Manna C, Galletti P, Cucciolla V, et al. Olive oil hydroxytyrosol protects human erythrocytes against oxidative damage. J Nutr Biochem. 1999; 10:159–165.
68. Visioli F, Galli C. The role of antioxidants in the

Mediterranean diet. Lipids. 2001;36(suppl):S49–S52.
69. Saija A, Trombetta D, Tomanio A, et al. In vitro evaluation of the antioxidant activity and biomembrane interaction of the plant phenols oleuropein and hydroxytyrosol. Int J Pharm. 1998;166:123–133.
70. Aeschbach R, Loliger J, Scott BC, et al. Antioxidant actions of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem Toxic. 1994;32:31–36.
71. Briante R, Febbraio F, Nucci R. Antioxidant properties of low molecular weight phenols present in the Mediterranean diet. J Agric Food Chem. 2003;51:6975–6981.
72. Manna C, D'Angelo S, Migliardi V, et al. Protective effect of the phenolic fraction from virgin olive oil against oxidative stress in human cells. J Agric Food Chem. 2002;50:6521–6526.
73. Quiles JL, Farquharson AJ, Simpson DK et al. Olive oil phenolics:effects on DNA oxidation and redox enzyme mRNA in prostate cells. Brit J Nutr. 2002;88:225–234.
74. Deiana M, Aruoma OI, Bianchi MDLP, et al. Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. Free Rad Biol Med. 1999;26:762–769.
75. Fabiani R, De Bartolomeo A, Rosignoli P, et al. Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. Eur J Cancer Prev. 2002;11:351–358.
76. Stupans I, Murray M, Kirlich, A, et al. Inactivationof cytochrome P450 by the food–derived complex phenol oleuropein. Food Chem Toxic. 2001;39:1119–1124.
77. Kohyama N, Nagata T, Fujimoto S, et al. Inhibition of arachidonate lipoxygenase activities by hydroxytyrosol, a phenolic compounds from olives. BioSci Biotech Biochem. 1997;61:347–150.
78. de la Puerta R, Gutierrez VR, Hoult JRS. Inhibition of leucocyte 5-lipxogenase by phenolics from virgin olive oil. Biochem Pharmacol. 1999;57:445–449.
79. Shureiqi I, Lippman,SM. Lipoxygenase modulation to reverse carcinogenesis. Cancer Res. 2001;61:6307–6312.
80. Montedoro G, Servili M, Baldioli M, et al. Simple and hydrolyzable phenolic compounds in virgin olive oil. I. Their extraction, separation and quantitative and semiquantitative evaluation by HPLC. J Agric Food Chem. 1992;40:1571–1576.
81. Mosca L, De Marco C, Visioli F, et al. Enzymatic assay for the determination of olive oil polyphenol content:assay conditions and validation of the method. J Agric Food Chem. 2000;48:297–301.
82. Tsimidou M, Papdopoulos G, Boskou D. Phenolic compounds and stability of virgin olive oil: part 1. Food Chem. 1992;45:141–144.
83. Gutfinger T. Polyphenols in olive oils. Journal of the American Oil Chemists' Society. 1981;58:966–968.