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Nutritional and Biological Properties of Extra Virgin Olive Oil

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The nutritional benefits generally recognized for the consumption of extra virgin olive oil (EVOO) are based on a large number of dietary trials of several international populations and intervention studies. Unfortunately, many authors in this field used questionable analytical methods and commercial kits that were not validated scientifically to evaluate the complex bioactive constituents of EVOO and lipid oxidation and decomposition products. Many questionable antiradical methods were commonly used to evaluate natural polyphenolic antioxidants, including an indirect method to determine low-density lipoprotein (LDL) cholesterol. Extensive differences were observed in experimental design, diet control, populations of different ages and problems of compliance intervention, and questionable biomarkers of oxidative stress. Analyses in many nutritional studies were limited by the use of one-dimensional methods to evaluate multifunctional complex bioactive compounds and plasma lipid profiles by the common applications of commercial kits. Although EVOO contains polyphenolic compounds that exhibit significant in vitro antioxidant activity, much more research is needed to understand the absorption and in vivo activity. Many claims of in vivo human beneficial effects by the consumption of EVOO may be overstated. No distinctions were apparently made between in vivo studies based on general health effects in large populations of human subjects and smaller scale well-controlled feeding trials using either pure or mixtures of known phenolic constituents of EVOO. More reliable protocols and testing methods are needed to better validate the complex nutritional properties of EVOO.

KEYWORDS: Extra virgin olive; nutritional benefits; methods; LDL oxidation; antioxidants; phenolic compounds; cholesterol; absorption; metabolism

INTRODUCTION

Evidence that the rate of cardiovascular deaths is lower in Mediterranean countries suggests that the beneficial effects of olive oil may not be related only to the known quantitative changes in plasma lipoproteins but also to other antiatherogenic factors. The chemical composition of extra virgin olive oil (EVOO) contributes to daily requirements of essential fatty acids and active antioxidant nutrients in vitamin E deficiency. This particular and well-balanced composition (oleic acid and minor components in an ideal ratio) may have significant effects in human clinical nutrition (*1*). Wide ranges of fatty acid compositions are reported for olive oils due to diverse environmental and cultivar characteristics, with the four major fatty acids ranges of 16:0, 7.8−18.8%; 18:1, 58.5−83.2%; 18:2, 2.8−21.1%; and 18:3, 0.42−1.9% (*2*). Because the level of saturated fatty acid content is generally below 10%, olive oil meets current dietary advice in terms of minimal saturated fatty acid intake.

Over the past three decades nutritional and epidemiological studies have provided accumulating evidence that the consumption of virgin olive oil (VOO) contributes to health benefits that can be attributed to many phenolic antioxidants, including mainly tyrosol (TYR) and hydroxytyrosol (HT) and their derivatives (aglycons of oleuropein and ligstroside, diacetoxy and dialdehydic forms of the aglycons), HT acetate, the lignans pinoresinol and 1-acetoxypinoresinol, luteolin, apigenin, and phenolic acids (*2*; see also ref *3*, Figure 1). Although commercial virgin olive oils contained higher levels of phenolic antioxidants than refined olive oils, their oxidative stability was significantly decreased by high initial peroxide values (15.6 and 32.5) (see ref *4*, Table 8.6, p 196).

A review of the extensive literature (*3*) on EVOO adulteration, oxidative stability, and antioxidants concluded that more reliable chemical and instrumental methods are needed to better understand the complex reactions involved in lipid oxidation and antioxidant chemistry and to detect adulteration with cheaper vegetable oils and deodorized oils. The many claims regarding the nutritional benefits of olive oils in the Mediterranean diet may be exaggerated without a better understanding on absorption and bioavailability of EVOO with better controlled human studies.

Although natural antioxidants of olive oils are multifunctional, many one-dimensional methods have been applied to evaluate their activity. A large number of protocols have been used to measure antiradical activity of these antioxidants by using free radical trapping methods such as DPPH (diphenyl-picrylhydrazyl radical), TEAC (Trolox equivalent antioxidant capacity), ORAC (oxygen radical absorbance capacity), FRAP (ferric reducing antioxidant power), and ABTS [(2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonate)], which are questionable because they are not specific

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and do not use suitable substrates to determine the protective properties of the antioxidants (see ref *4*, Table 9.19, p 250, and ref *5*, Table 4.3, p 84).

OXIDATIVE STRESS

Several intervention studies supported the cardiovascular protective effects of the Mediterranean diet that includes VOO as the main fat consumed and related these effects to high levels of oleic acid and/or antioxidants. Many feeding studies have been published to evaluate the effects of olive oil diets on oxidative stress and susceptibility of human LDL and plasma (**Table 1**). Favorable effects of minor components of VOO were evaluated on fasting and postprandial lipid profiles and on LDL composition and susceptibility to Cu-catalyzed oxidation in vitro (*6*). The study was performed with males having normal lipid profiles, comparing diets of VOO with oleic acid rich sunflower oil, containing 75.53 and 77.87% oleic acid, respectively, and showed no significant difference in fasting and postprandial states. Only a few minor variations were observed in LDL composition in postprandial lipemia (excess fat in blood), but the VOO diet resulted in a small but insignificant decrease in conjugated diene formation.

In another study (*7*) of male subjects with peripheral vascular disease, the major fatty acid profiles in plasma and LDL were not different after the consumption of EVOO and refined OO (ROO) with different antioxidant profiles (300 vs 200 mg/kg α-tocopherol, 800 vs 60 mg/kg total phenols). However, the rate of Cu-catalyzed oxidation evaluated by questionable TBARS (thiobarbituric acid reactive substances) and total LDL taken up by macrophages were significantly greater after the intake of ROO compared to EVOO. A further study of healthy men and women (*8*) showed no differences in resistance of LDL and HDL to oxidation between two diets of VOO containing different levels of total phenols (308 vs 43 mg/kg). Unfortunately, questionable markers of oxidation were used on the basis of expedient commercial kits, the questionable measure of lipid oxidation TBARS, and antiradical unspecific FRAP test (*4*,*5*). An intervention dietary study using young men and women subjects (*9*) showed that a high-carbohydrate diet and a Mediterranean diet decreased LDL-cholesterol and HDL-cholesterol determined by indirect methods (**Table 1**). The authors concluded that both diets can improve glucose metabolism in these young subjects. A study with healthy male and female Greek smokers (*10*) comparing the effect of two levels of phenol content in EVOO (43 and 308 mg/kg) found no significant differences in plasma resistance to Cu-mediated oxidation in fasting plasma, concentration of protein carbonyl, lipid oxidation, and questionable MDA and FRAP tests in plasma.

A study based on the oxidative/antioxidant status of healthy males (*11*) compared olive oils with different phenolic contents (10, 133, and 486 mg/kg total phenols). Dose-dependent changes included decreased plasma oxidized LDL, oxidized mitochondria DNA, and MDA in urine and increased HDL cholesterol and glutathione peroxidase activity. Like many other studies in **Table 1**, the authors used a questionable enzymatic kit to measure oxidized LDL in plasma by ELISA, urinary MDA, and LDL-cholesterol. A similar study with nonsmoking healthy males (*12*) compared the effects of olive oils with different phenol contents increasing from 0 to 150 mg/kg. Urinary TYR and HT increased, in vivo plasma oxidized LDL decreased, and ex vivo resistance to LDL oxidation and HDL cholesterol levels increased with the phenol content of olive oil administered. Questionable commercial kits were used for oxidized LDL by ELISA and antigen antibody.

Using mildly dyslipidemic patients to compare the vasoprotective potential of EVOO with that of refined olive oil (ROO) (*13*), no effects were found on plasma lipid and lipoprotein profiles. On the other hand, the feeding of EVOO decreased serum thromboxane B_2 formation and urinary isoprostane excretion used as markers of cardioprotective potential and vascular function and increased questionable antioxidant capacity of plasma based on the nonspecific reduction of cupric to cuprous ions. A similar study using stable coronary heart disease patients (*14*) compared the antioxidant and antihypertensive effects of VOO and ROO. Intervention with EVOO resulted in lower plasma oxidized LDL and lipid peroxide levels (based on sandwich ELISA) and lipid peroxides (based on questionable TBARS), together with higher antioxidant activity based on the artificial radical 2,2′-azinobis(3-ethylbenzthiasoline) sulfonate (ABTS) and glutathione peroxidase.

A large feeding trial for the EUROLIVE study group with healthy male subjects (*15*) compared diets using olive oils of different phenol contents (2.7, 164, and 366 mg/kg). A linear increase in HDL cholesterol levels with phenol contents was observed, accompanied with a linear decrease of markers of oxidative stress. Statistical changes in conjugated dienes, hydroxy fatty acids, and oxidized LDL were not significant ($p = 0.011$, 0.038, and 0.014, respectively). Unfortunately, no references were provided for any of the methods used for oxidative damage of lipids or endogenous and exogenous antioxidant status.

Another study led by the same lead author (*16*) was aimed at examining the role of the same olive oils on postprandial (after a big meal) oxidative stress and the antioxidant content of LDL. The low-phenolic diet resulted in a decrease and oxidation of LDL phenolics by impairing endothelial functions referred to as *postprandial* oxidative stress. The levels of F_2-isoprostanes (cyclic oxidation products of arachidonic acid), oxidized LDL, and antibodies against oxidized LDL decreased directly with the levels of total phenols in the diets. The degree of LDL oxidation decreased as the phenolic content of the olive oil administered increased. The same beneficial effects were obtained by adding suitable sources of antioxidants such as red wine, vitamin C, or "antioxidant" drugs. This evidence supports the conclusion that phenolic antioxidants in olive oil can protect LDL against oxidation and can modulate the oxidative/antioxidant balance in plasma and LDL. However, this study, like many others in **Table 1**, had limitations due to the measurement of oxidized LDL in plasma with a complex sandwich ELISA procedure using commercial complex antibodies and that of the plasma lipid profile by another expedient commercial enzymatic kit.

Oxidative DNA damage was evaluated in another trial with postmenopausal women by comparing high- and low-phenolic EVOO [592 and 147 mg/kg total phenol (*17*)]. Oxidative DNA damage was 30% lower after the consumption of the high-EVOO compared to the low-EVOO treatments on the basis of a comet assay in periperipheral blood lymphocytes. Changes in plasma antioxidant capacity by the questionable ABTS assay were not significantly different after consumption of high- and low-EVOO diets. In another large human study (*18*), DNA and RNA oxidation was evaluated in northern, central, and southern European populations consuming olive oils with low, medium, and high phenolic contents. Urinary excretion of oxidation products of guanine (8-oxo, -guanine, -guanosine, and deoxyguanosine) were not different when the different olive oils were compared. A 13% reduction of DNA oxidation was observed on the basis of urinary 8-oxodeoxyguanosine, showing that although ingestion of olive oil is beneficial in reducing DNA oxidation, this effect is not due to the phenolic content in the olive oils.

A large clinical trial to assess in vivo lipoprotein oxidation used subjects of high cardiovascular risk (*19*). A traditional Mediterranean diet (TMD) was tested for the prevention of coronary heart disease by comparing a low-fat diet used as control with two

Table 1. Effects of Olive Oil (OO) Diets on Oxidative Stress and Susceptibility of Human LDL and Plasma[a]

system	protocol	diet	analyses plasma	results	limitations	ref, year
Cu-oxidized LDL in blood, postprandial lipemia	10 healthy subjects	VOO vs oleic rich sunflower oil	CD, TC, LDL-C, TG, apo B	no significant difference	commercial enzymatic kits: TC, TG, Chol, PL[b]	*6*, 1998
Cu-oxidized LDL in blood	24 subjects	EVOO vs refined OO	total cholesterol	LDL oxidation higher with refined OO	commercial kits for chol, TAGs, TBARS[c]	*7*, 1999
Cu-oxidized LDL in blood	46 healthy subjects	VOO rich vs poor, low vitamin E	CD, plasma MDA, TBARS, FRAP, protein carbonyls, lipid-OOH	no effect on LDL and HDL oxidation	commercial kits for lipid-OOH, serum uric ac, TBARS, FRAP $Fe^{3+} \rightarrow Fe^{2+}$[d]	*8*, 2001
glucose metabolism	59 healthy males or females	low fat, high carbohydrate	LDL and HDL cholesterol	both diets decreased LDL-Chol	LDL-chol by indirect method[e]	*9*, 2001
total plasma Cu oxidation	25 healthy males and females	low and high phenol OO	CD, MDA, lip-OOH, ELISA protein carbonyls FRAP	no significant differences in oxidation markers	commercial kits for plasma lipid-OOH etc., serum uric ac, MDA, FRAP[d]	*10*, 2002
plasma-oxidized LDL	12 healthy males	low, moderate, high phenol-OO	oxLDL, MDA in urine, lipid-OOH, phenols in plasma, urine	decreased in oxidized markers, increased in phenols	commercial enzymatic kits, oxLDL by ELISA, urinary MDA, LDL-C [e,f]	*11*, 2004
in vivo oxidized LDL, serum antibodies	30 healthy nonsmoking males	refined, common, VOO	oxLDL, resistance of LDL oxidation	decrease in vivo oxLDL, increase ex vivo LDL stability	commercial kits for oxLDL, ELISA antigen antibody[g]	*12*, 2004
in vitro antioxidant plasma and serum TXB_2	22 mildly dislipidemic patients	ROO, EVOO	plasma antioxidant capacity	TXB_2 decrease, antiox capacity increase	commercial kit for $Cu^{2+} \rightarrow Cu^{+}$ TXB_2 qestionable antiox capacity[h]	*13*, 2005
plasma and serum LDL	40 stable CHD patients	ROO, VOO differences in PC levels	lipid-OOH, GSH peroxidase, TBARS, total antiox status	lower plasma oxLDL, Lip-OOH higher GSH peroxidase	sandwich ELISA, oxLDL, TBARS, ABTS radical [i]	*14*, 2005
lipid oxidative damage	200 healthy males	low, moderate, high phenol OO	oxLDL, OH FA, isoprostane, CD, oxidative damage, antiox status	↑HDL-C↓total-C/HDL-C and oxid stress markers	enzyme immunoassay antiox status by HPLC, LDL-C by indirect method[e]	*15*, 2006
postprandial oxidative stress	12 healthy males	low, moderate, high phenol OO	LDL antiox content, tyrosol, OH tyrosol, phenolic cpds	phenolic cpds absorbed in postprandial state	oxLDL, ab-oxLDL by ELISA TC; HDL-C, TG by enzymatic kit [i,j]	*16*, 2006
DNA oxidative damage	10 postmenopausal females	high and low-EVOO	DNA breaks + oxidized bases by Comet assay	30% lower DNA oxid damage with high vs low EVOO	antiox capacity of plasma by ABTS, Comet assay[k]	*17*, 2006
DNA and RNA oxidation	344 subjects	low, moderate, high phenol OO	8-hydroxylated guanine, guanosine, deoxyguanosine	no difference in urinary RNA and DNA oxid prod	HPLC/MS of urine samples (no details)	*18*, 2007
oxidized LDL	372 subjects	TMD + VOO vs TMD + nuts vs low fat diet	MDA in mononuclear cells of oxid LDL	oxLDL↓with TMD + VOO, TMD + nuts, no change with low-fat diet	questionable MDA, commercial kit immunosorbent assay, GSH-PX [e,l]	*19*, 2007
inflammatory and oxidative stress markers	12 normolipidemic males	EVOO, OO, corn oil	TXB_2, LTB_4 serum antiox capacity, $Cu^{2+} \rightarrow Cu^{+}$	EVOO → less inflammation OO or CO → no effect	FOX reagent commercial kit[m]	*20*, 2007

Table 1. Continued

system	protocol	diet	analyses plasma	results	limitations	ref, year
LDL fatty acid composition	200 healthy males	low, moderate, high phenol OO	HDL, oxid LDL, isoprostanes, uninduced CD in serum LDL	inverse relationship of 18:1/18:2 and oxid stress	unspecified methods for TC HDL-C, TAGs, LDL-C by indirect method	*21*, 2008
LDL, oxidized LDL	17 diabetic vs 23 healthy elderly subjects	diets rich in VOO vs sunflower oil	systolic blood P LDL-C and HDL-C, lipid profiles, LDL oxidation	VOO ↓ blood P and LDL oxidn in diabetic patients	LDL oxidn with Fe in PO_4 buffer ascorbate	*22*. 2009
plasma LDL	36 nonsmoking males	VOO (629 mg/L phenol) vs ROO (0 mg/L phenol)	oxLDL (by ELISA), serum CD, plasma OH- FA	oxLDL, CD and OH-FA decreased only w/VOO	dietary intake based on self-reporting	*23*, 2010
endothelial function, oxid stress, inflammation	37 healthy volunteers	vegetable oils vs EVOO	lipidoperoxides, vasc adhesion, blood P	all oils → no effect on inflammation, oxid stress	questionable commercial kits, PEROX, sVCAM-1[n]	*24*, 2010

[a] Abbreviations: ↑, increase; ↓, decrease; →, gives; Ab-oxLDL, antibodies against oxidized LDL; ABTS, 2,2′-azinobis(3-ethylsulfonate); antiox, antioxidant; CD, conjugated dienes; CHD, coronary heart disease; Chol, cholesterol; EVOO, extra virgin olive oil; FRAP, ferric reducing ability of plasma; GSH, glutathione; GSH-Px, GSH peroxidase; lipid-OOH, lipid hydroperoxides; HDL-C, high-density lipoprotein cholesterol; HT, hydroxytyrosol; LDL, low-density llipoproteins; LTB_4, leukotriene B_4; LDL-C, LDL cholesterol; MDA, malondialdehyde; OLAB, oxidized LDL serum antibodies; OH-FA, hydroxyl fatty acids; oxLDL, oxidized LDL; PC, phenolic compounds; PV, peroxide value; PVD, peripheral vascular disease; ROO, refined olive oil; TAGs/TG, triacylglycerols; TAS, total antioxidant status; TBARS, thiobarbituric acid reactive substances; TC, total cholesterol; TMD, traditional Mediterranean diet; TXB_2, thromboxane B_2; TYR, tyrosol; VOO, virgin olive oil. [b] Abbot Diagnostic VP analyzer and HDL-C kits (Boehringer, Mannheim, Germany). [c] Preciset Cholesterol and Peridochrom Trigliceridos PAP (Boehringer, Mannheim, Germany), precipitation with polyanions, TBA measuring MDA is questionable. [d] MDA for plasma is questionable, K-assay LPO-CC kit (Kamiya Biomedical Co, Seattle, WA), serum uric acid by UA kit (Boehringer, Mannheim, Germany). [e] Indirect method without using a preparative ultracentrifuge to estimate LDL cholesterol using the Friedewald formula (Friedwald et al. *Clin. Chem. B* **1972**, *18*, 499−502). [f] MDA in urine and oxidized LDL in plasma by ELISA, K-assay, LPO-CC kit, enzymatic methods for glutathione peroxidase and reductase (Randox Laboratories). [g] In vivo oxidized LDL was determined in plasma by a sandwich ELISA procedure (Mercodia AB, Uppsala, Sweden); oxLDL by ELISA antigen, antibodies (OLAB, Biomedica, Vienna, Austria). [h] Total antioxidant capacity of plasma by reduction of $Cu^{2+} \rightarrow Cu^{+}$ (Oxis Research, Portland, OR), TXB_2 in serum by immunoassay (Cayman Chemicals, Ann Arbor, MI). [i] Oxidized LDL (oxLDL) in plasma by sandwich ELISA and antibody for oxLDL bound on solid phase (Ab-oxLDL, Mercodia AB); TC, HDL-C, and TG levels by enzymatic kits (Hofman-La Roche Diagnostic, Basel, Switzerland); TBARS, antiradical ABTS, and TAS by Cobas Mira plus analyzer (Randox Laboratories, Crumlin, Antrim, U.K.). [j] Unspecified methods for oxidative damage measured by enzyme immunoassay, isoprostane, serum LDL cholesterol by GC-MS, and antioxidant status by HPLC. [k] Antioxidant capacity by ATBS (TAS, Randox Laboratories. Ltd., Crumlin), GSH-Px (ABX diagnostics, Madrid, Spain), Comet assay to measure DNA breaks and oxidized bases. [l] Serum GSH-Px by spectrophotometry (Randox Laboratories, Crumlin, Northern Ireland). [m] Antioxidant capacity method claimed to be "established." Oxidative stress by Metra Creatinine Assay kit (Quidel, San Diego, CA). [n] Total lipid PEROX by commercial photometric technique (kit by ImmunDiagnostik, Bensheim, Germany), sVCAM-1 serum levels (ELISA kit by R&D Systems, Wiesbaden, Germany).

TMD diets: TMD + VOO or TMD + nuts. After a 3 month intervention, oxidized LDL decreased significantly in both groups, without changes in the control low-fat diet. Circulating oxidized LDL was measured by an enzyme-linked immunosorbent assay using a commercial antibody with no details. The questionable MDA test was used in mononuclear cells isolated from fresh blood measured by HPLC. A much smaller study of postprandial anti-inflammatory and antioxidant effects of EVOO (*20*) evaluated changes in inflammatory markers thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4), and oxidative stress markers (urinary H_2O_2 and serum antioxidant capacity). The inflammatory markers decreased significantly together with a corresponding increase in antioxidant capacity after feeding EVOO, but not plain olive oil or corn oil consumption. Antioxidant capacity was based on the nonspecific and reduction of Cu^{2+} to Cu^{+}, and urinary H_2O_2 was determined by a nondescript commercial and expedient FOX reagent.

Another large EUROLIVE study examined the relationship between changes in fatty acid composition of LDL and lipid oxidation damage after sustained consumption of olive oil (*21*). The oleic acid concentration increased and those of linoleic and arachidonic acids decreased in LDL after olive oil intake. An inverse relationship was obtained between oleic/linoleic acids and biomarkers of oxidative stress, including circulating oxidized LDL measured by an unspecified enzymatic immunoassay. An intervention study (*22*) showed that a diet rich in olive oil compared to sunflower oil (75 vs 25% oleic acid and 5 vs 65% linoleic acid, respectively) protected LDL from oxidation in elderly type 2 diabetic subjects compared to healthy elderly controls. However, LDL oxidation in this study was catalyzed by an excessive iron concentration that, in the presence of ascorbate, is reduced to the more catalytically active ferrous state.

The effect of VOO containing 629 mg/L phenols was compared with a similar refined olive oil (ROO) containing no phenols in another EUROLIVE study of plasma LDL oxidation in males (*23*). The dietary intake of the subjects was based on self-reporting. Oxidation markers including oxidized LDL, conjugated dienes, and hydroxyl fatty acids decreased after VOO ingestion, but they were not affected after the consumption of ROO. The authors concluded that after sustained consumption, the phenol concentration of the consumed OO modulates the phenolic metabolite content of LDL and supports the in vivo antioxidant role of phenolic compounds.

The effects of consuming VOO were compared with those of consuming maize oil, cod liver oil, and soy oil in a study of endothelial function and oxidative stress in healthy young subjects (*24*). On the basis of measurements of total lipid peroxides, vascular adhesion molecules, and blood pressure, this investigation concluded that acute consumption of maize oil blunts endothelial function in contrast to a slight improved endothelial function with codfish and soy oil and only neutral effects with olive oil. Unfortunately, the authors used a questionable commercial kit for total lipid PEROX and ELISA to measure vascular cell adhesion molecules.

NUTRITIONAL ASPECTS

The consumption of olive oils has been associated with many desirable nutritional properties including lower incidence of coronary heart disease and cancer. The authors of several studies of EVOO in the literature often claimed desirable in vivo nutritional benefits of phenolic antioxidant constituents based mainly on population studies and statistical evaluations of their results. However, a better mechanistic understanding of the nutritional effects of olive oils is also required on the basis of absorption and metabolic studies with pure and known mixtures of olive oil antioxidants.

In addition to their antioxidant effects, flavonoids in olive oil and other fruit sources may have a multitude of beneficial activities that can be attributed to nonantioxidant chemopreventive activities in the gastrointestinal (GI) tract (**Table 2**). Other biological effects of flavonoids have now been recognized after they are absorbed in vivo and converted into metabolites such as glucoronides, sulfates, and *O*-methylated derivatives with reduced antioxidant activity (*25*). Enzymatic degradation occurs in the colon, where microflora hydrolyze flavonoids into simple phenolic acids, which are absorbed and further metabolized in the liver. Flavonoids have also been shown to modulate the expression of γ-glutamylcysteine synthetase, an important enzyme in cellular antioxidant defenses and detoxification of xenobiotics (*26*). This enzyme catalyzes the synthesis of glutathione (GSH), which is considered to be an important endogenous biological antioxidant in cells. Polyphenol-mediated regulation of GSH alters cellular processes by redox regulation of transcription factors and enzymes catalyzing signal transduction. Flavonoids in fruits and vegetables may modulate the intracellular GSH concentration by regulating redox switching of many protein cellular functions.

Minor components of VOO (about 1−2%) have been associated with beneficial cardiovascular effects (*27*). These constituents in olive oil include phenolic compounds (TYR, HT, caffeic acid, and oleuropein), tocopherols, sterols (β-sitosterol, campesterols, Δ7-stigmasterol, brassicasterol), and hydrocarbons (squalene, β-carotene, and polycyclic aromatic hydrocarbons). Many of these compounds have been shown to have anti-inflammatory and hypolipidemic properties in addition to their antioxidant activities. Endothelial function measured by low-mediated dilation was found to decrease after the consumption of olive-oil rich meals compared to canola oil and salmon. Olive oil consumption was inversely associated with blood pressure and contributed to the reduction of the oxidative modification of LDL and uptake of LDL by macrophages that initiate the formation of fatty streaks leading to atherosclerosis. The protective roles of olive oil phenolic compounds against LDL oxidation in vitro were found to be equivalent to that of vitamin E.

In addition to their antioxidant activities, most of these phenolic compounds in EVOO have anti-inflammatory and hypolipidemic properties. The mechanism of action for modulating their endothelial activity involves the release of nitric oxide, prostaglandins, leukotrienes, and adhesion molecules as well as activation of nuclear factor κB by reactive oxygen species. Although the relative absorption of polyphenols of VOO is controversial, it is mostly converted into conjugated derivatives of glucuronic acid in plasma, but intervention studies reported reduced oxidative stress in healthy and dyslipidemic subjects.

In a study of the gastrointestinal fate of olive oil polyphenols (*28*), model studies were carried out with cell cultures under gastric juice conditions (pH 2, 37 °C, up to 4 h) simulating the stomach. HPLC analyses showed a time-dependent hydrolysis of conjugated olive oil polyphenols and the formation of the main components HT and TYR. Transport and metabolic experiments with a perfused rat intestinal model showed that oleuropein was not transferred across small intestinal segments, whereas HT and TYR were rapidly absorbed. After perfusion, free HT, methylated HT, and the respective glucuronides were detected. Under more complex in vivo conditions than used in this model, the presence of food enzymatic degradation of food protein may also occur. The authors concluded that the small intestine is another major site of absorption of olive oil phenols that results in increasing concentration of HT and TYR in plasma initially and in urine metabolized after a few hours into *O*-methylated HT,

Table 2. Nutritional Effects of Polyphenolic Compounds in Olive Oils (OO)

antioxidants + mediators	conjugated metabolites	cellular and pharmacological effects	disease preventions	deleterious oxidation products and negative effects	ref, year
quercetin, catechin, epicatechin	*O*-methyl, glucuronides, sulfates, adducts	kinase, lipid and protein kinase signals, signal transduction	cancer, inflammation, neurodegeneration	semiquinone and quinones, apoptosis	*25*, 2004
polyphenols + glutathione (GSH)	γ-glutamyl synthetase	detoxification of xenobiotics	gene regulation	xenophobic prooxidants, reduce cellular GSH	*26*, 2005
hydroxytyrosl (HT), tyrosol (TYR), oleuropein	*O*-methyl, glucuronides, GSH conjugate	anti-inflammatory, inhibition of cytokine and eicosanoid monocyte adhesion of endothelial cells	increased bioavailability of phenols, reduced blood pressure	decreased antioxidant activity, HOSO cannot reduce high blood pressure	*27*, 2006
olive oil phenolics	TYR and HT metabolites	postprandial lipemia, lower blood pressure	inhibition of LDL and DNA oxidation	acid conditions in GI tract may increase levels of HT and TYR in small intestine	*28*, 2006
flavonoids, lignans, simple phenolics,	*O*-glucuronides of HT are stronger radical scavengers than HT	antioxidant and nonantioxidant effects	colon cancer, detoxification of enzyme and immune systems	controversial effects of EVOO in real-life doses, more controlled studies needed	*29*, 2007
HT and TYR	HT and TYR metabolized to o-Me-HT, HT-GSH, and glucuronides	prevention of colonic cancer, direct antioxidant effects in GI tract + nonantioxidant effects	control growth, differentiation and metathesis of cancer cells	insufficient in vivo information on effects of olive polyphenols on normal cells in GI tract	*30*, 2009

glucuronidated HT and TYR, and glutathionylated HT in the small intestine.

A large number of biological and nutritional effects of olive oil have been reviewed for lipoprotein metabolism, oxidative damage, inflammation, endothelial dysfunction, blood pressure, and thrombosis. In a previous review regarding the effects of OO on the cardiovascular system (*29*), a majority of 11 references reported no effects on lipid and DNA damages. However, questionable methods were used in many of these papers including antioxidant capacity based on undefined and expedient commercial kits for lipid hydroperoxides, MDA, and FRAP. Most of these commercial kits have not been validated scientifically.

The biological effects of OO consumption are mainly linked to the direct or indirect antioxidant activity of the phenolic constituents and their metabolites that become concentrated in the GI tract (*30*). Olive oil phenolics can be partially hydrolyzed after ingestion under the acidic conditions of the stomach. Free HT and TYR are produced from the aglycone secoiridoids, but glucosides of oleuropein are absorbed intact without hydrolysis. The secoiridoids absorbed intact in the small intestine are degraded mainly into HT by colonic microflora. Other phenolic compounds and their metabolites are considered to be largely responsible for the anticancer activity of EVOO. Corresponding effects in modulating colon cancer are also attributed to the influence of olive polyphenolics to cell proliferation, apoptosis, and arachidonic acid metabolism in cancer cells. Although phenolic compounds from OO, grapes, and other fruits are recognized to exercise important protective antioxidant effects by directly reacting with reactive oxygen species in the GI tract (*31*), not much is known yet on the nutritional effects of the metabolites derived from flavonoids.

Many studies have reported the neuroprotective, cardioprotective, and chemopreventive actions of dietary flavonoids (*25*). It is now becoming apparent that flavonoids, and in vivo metabolites, not only act as conventional hydrogen-donating antioxidants but may also exert "non-antioxidant" activities in cells through actions at protein kinases and lipid signaling pathways. Inhibitory or stimulatory actions at these pathways may affect cellular function by altering the phosphorylation of proteins and modulating gene expression. The influence of flavonoid metabolites on these properties may be keys to their activities as anticancer agents, cardioprotecting agents, and inhibitors of neurodegeneration.

The bioavailability and bioefficacy of phytochemicals can be improved significantly by new delivery systems using nanoemulsions consisting of extremely small droplets ranging between 1 and 100 μm (*32*). Improved in vitro and anticancer activities of phytochemicals are claimed to be achieved by the use of biopolymer micelles that are more soluble and dispersible than conventional emulsions. Liposome-encapsulated antioxidants have also been used to study in vitro and in vivo effects on cell growth and apoptosis in cancer. This novel approach may be promising in future research using polyphenolic compounds of EVOO.

The authors of several references in **Table 1** used questionable and expedient methods to evaluate lipid oxidation and antioxidants. Commercial kits without detailed validation were used in many studies to determine enzyme activity (*6*, *13*, *19*, *24*), cholesterol (*6*, *7*), plasma lipid hydroperoxides (*8*, *10*), oxidized LDL in plasma by ELISA (*11*, *12*, *14*, *16*), and total lipid peroxides PEROX (*26*). Determinations of TBARS are notoriously unspecific (*4*, *5*) and unsuitable to determine complex LDL oxidation (*7*, *8*, *14*). LDL cholesterol was determined in many studies by an indirect method without isolating LDL by using a preparative ultracentrifuge according to accepted methods (*9*, *11*, *15*, *19*, *21*). A large number of authors in many papers listed in this table

performed in collaboration with many medical and pharmaceutical investigators from various European Union countries and contributors to large trials and feeding study groups: SOLOS (*11*, *12*, *14*), VOLOS (*13*), EUROLIVE (*15*, *16*, *18*, *21*), and PREDIMED (*16*, *19*). Extensive differences were noted in experimental design, diet control, populations of different age and problems of compliance intervention, and questionable biomarkers of oxidative stress. Many studies in **Table 1** were also limited by using undefined sandwich ELISA procedures, commercial complex bioactive compounds, and plasma lipid profiles by commercial kits. Unfortunately, some of the feeding studies provided excessive details on the questionnaire used for the participants and the statistical analyses but fewer details on the chemical analyses used. Many claims in the literature of in vivo human effects by consuming EVOO may be exaggerated. In vivo human studies that are based on general health effects in large populations should be distinguished with feeding trials of pure concentrates of phenolic constituents followed by testing plasma or tissue samples taken shortly after the consumption of different amounts of EVOO.

Many nutritional studies listed in **Table 2** dealing with the relative absorption of polyphenols of VOO may be controversial because they used model systems. Under more complex in vivo conditions, the presence of food enzymatic degradation of food protein may also occur. Unfortunately, only little information is available on the nutritional effects of the metabolites of flavonoid compounds. Many studies on the effects of OO on the cardiovascular system reported no effects on lipid and DNA damages, using nonspecific one-dimensional methods for "antioxidant capacity", questionable markers of oxidation, and commercial kits that have not been validated scientifically. Several more specific and reliable methods should be used to obtain chemical information that can be directly related to oxidative damage of biological systems.

More research is needed to better understand the bioavailability of dietary phenolic antioxidants and other minor constituents of EVOO, their interactions with other food components and metabolism, and other factors affecting their absorption in vivo (*33*). More valid protocols and testing methodology in feeding human studies are required to better establish the health and nutritional benefits of olive oils with more valid chemical methods and less reliance on expedient commercial kits to evaluate their complex nutritional properties.

LITERATURE CITED

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