

Original Communication

Antioxidant Activity of Olive Polyphenols in Humans: a Review

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Abstract: *In vitro* and animal studies show that polyphenols from olives have potent antioxidant activities; 50 % of the phenolic compounds contained in olives and virgin olive oil are hydroxytyrosol and derivatives thereof. Hydroxytyrosol is the major olive polyphenol consumed and well absorbed in humans. It is considered to have the highest antioxidant potency compared to the other olive polyphenols. Review of the human intervention studies showed that olive polyphenols decreased the levels of oxidized-LDL in plasma and positively affected several biomarkers of oxidative damage. The antioxidant effects of olive polyphenols on low-density lipoprotein (LDL) oxidation are observed after a dietary intake of about 10 mg per day. The overall evidence from *in vitro* assays, and animal and human studies support the antioxidant effect of olive polyphenols. However, further larger human studies are needed to clarify the effect of olive polyphenols on markers of oxidative stress, particularly DNA damage and plasma isoprostane levels.

Key words: olive, polyphenols, phenolics, hydroxytyrosol, antioxidant, human

Introduction

The Mediterranean diet rich in fruits and vegetables has been associated with a healthy lifestyle and longevity [1–3]. It is, among its other features, characterized by a high consumption of olives and olive oil. Epidemiological and intervention studies point at beneficial effects of the Mediterranean diet to reduce chronic diseases, particularly cardiovascular diseases [1–8]. Recently, M.-I Covas published an excellent review of the effects of olive oil and olive oil minor components on the cardiovascular system [9]. Olive products are rich sources of polyphenols; the major representatives are hydroxytyrosol and derivatives thereof [10–12] with antioxidant, anti-thrombotic,

anti-atherogenic, and anti-inflammatory properties [11,12]. Hydroxytyrosol is an ortho-diphenol and is the strongest radical-scavenger *in vitro* among all the olive polyphenols [11–13]. It is considered to be one of the main components accounting for the beneficial properties of olive products. The content of olive polyphenols in table olives and olive oil, the two main olive based foods in the Mediterranean diet, is affected significantly by the type of treatment and the olive varieties. In table olives, the hydroxytyrosol content can range from about 400 mg/kg up to 1000 mg/kg for certain varieties [14,15]. Typically virgin olive oil contains about 15 to 20 mg/kg of hydroxytyrosol and refined olive oil less than 2 mg/kg [11]. The aim of this paper was to review the evidence

derived from the published human intervention studies for the antioxidant activity of olive polyphenols.

Background

Increased oxidative stress is implicated in the pathogenesis of many acute and chronic diseases and plays an important role in the aging process [16–19]. Oxidative stress occurs as a result of an imbalance between the oxidant and antioxidant systems of the body, which leads to excessive production of reactive oxygen species. Free radicals can damage various cellular components by targeting lipids, protein, and DNA. In the body the oxidative stress produced by free radicals is controlled by various endogenous enzymatic antioxidant systems, such as superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and by non-enzymatic antioxidants such as vitamins C and E, carotenoids, and polyphenolic compounds [20,21]. These compounds are key factors in preventing the deleterious effects of oxidative stress and the development of related chronic disorders such as cardiovascular and neurodegenerative diseases [22–24]. Therefore, they should be present in a healthy diet. The Mediterranean diet is particularly rich in antioxidants. Virgin olive oil with its high polyphenol content is the major source of fat in such diets, thus contributing significantly to the antioxidant protective effect of a typical Mediterranean diet [25].

The antioxidative properties of olive polyphenols *in vivo* will depend on the extent of their absorption and metabolism. Hydroxytyrosol in its free, secoiridoid, or conjugated form makes up about 50 % of the phenolic compounds in olives and virgin olive oil [14,26]. Oleuropein, a secoiridoid, is an ester of hydroxytyrosol and elenolic acid or its glycoside and can be hydrolyzed to hydroxytyrosol [11]. Visioli *et al.* [27] reported the first evidence that hydroxytyrosol and tyrosol are dose-dependently absorbed in humans and excreted in urine. The pharmacokinetics of hydroxytyrosol in human plasma was later described by Miro-Casas *et al.* [28]. Thus, hydroxytyrosol is dose-dependently absorbed in the human intestine, as indicated by the observed increase in plasma levels after dietary olive polyphenol intake [27–31]. Further studies are needed to evaluate whether hydroxytyrosol could accumulate in other tissues besides plasma after long-term consumption. The complex olive polyphenol conjugated forms are hydrolyzed in the stomach and intestine, resulting in an increase in the plasma levels of hydroxytyrosol and

tyrosol [32,33]. Corona *et al.* [32] showed that oleuropein is poorly absorbed from the small intestine, but does reach the large intestine where it is rapidly degraded by the microflora to yield hydroxytyrosol, which can then be absorbed. Thus, despite its poor bioavailability, oleuropein is metabolized into the aglycon hydroxytyrosol in the intestine and absorbed in the human body [30,32,33]. The antioxidant properties of the olive polyphenols have been extensively studied and their effects are described in numerous reviews [9–13,25,34–36]. Olive polyphenols are potent radical scavengers and they inhibit the oxidation of lipids and of low-density lipoprotein (LDL) particles; their antioxidative properties have been demonstrated both *in vitro* and *in vivo*. Hydroxytyrosol, an ortho-diphenol, is considered to be a potent antioxidant due to its two adjacent hydroxyl groups. Hydroxytyrosol inhibits copper-induced LDL oxidation [37] while a mono-phenol, such as tyrosol, has little antioxidant activity and does not protect LDL from chemically-induced oxidation. Therefore, the olive oil polyphenols with a catechol moiety, such as hydroxytyrosol or its derivatives, are considered to be the major antioxidant in olive products. Numerous *in vitro* studies support the antioxidant activity of hydroxytyrosol or its derivatives [38–49]. Rietjens showed that hydroxytyrosol efficiently protects vascular tissue against oxidative stress [50]. Hydroxytyrosol was also shown to reduce oxidative damage in intestinal epithelial cells [43], hepatocytes [41,43], and human erythrocytes [42,43]. Hydroxytyrosol is an efficient radical scavenger (superoxide anion, hydroxyl radical, peroxyxynitrite) and has metal-chelating capacities; it efficiently protects against LDL oxidation *in vitro* at relatively low concentrations. The data show that hydroxytyrosol is a potent inhibitor of lipid peroxidation, which is considered to be one of the main mechanisms of tissue damage by free radicals. Finally, the antioxidant properties of olive polyphenols were also demonstrated *in vivo* in animal models [51–54]. Thus, olive polyphenols and particularly hydroxytyrosol clearly can reduce oxidative damage *in vitro* and *in vivo* and protect cells from oxidative damage.

Antioxidant activity of olive polyphenols in humans

Identification of pertinent human studies

Literature search

The bibliographic databases PubMed and SciFinder® were searched for human studies related to the antioxidant properties of olive polyphenols using the terms hydroxytyrosol, oleuropein, olive and phenolic*, olive, and polyphenol*. We also performed a manual search of references listed in review articles or manuscripts.

Identification of pertinent studies and exclusion criteria

All identified human intervention studies related to the antioxidant properties of olive polyphenols were included in the analysis, unless the studies were primarily investigating the *ex vivo* susceptibility of LDL to oxidation. Studies dealing with *ex vivo* resistance of LDL to oxidation showed inconsistent results after consumption of olive polyphenols. On the contrary, olive polyphenols efficiently protect against LDL oxidation *in vitro* and *in vivo*. In *ex vivo* experiments, LDL is isolated from humans after olive polyphenol consumption, and subsequently the oxidizability of the LDL is determined *ex vivo*. Rietjens *et al.* [55] showed that the LDL isolation procedure substantially reduces the protective effect of hydroxytyrosol against LDL oxidation due to the loss of hydroxytyrosol from LDL during the isolation procedure. Such data help to explain the inconsistent results observed in *ex vivo* studies [55]. The *ex vivo* isolation procedure for LDL will remove the water-soluble olive polyphenolics (hydroxytyrosol and related compounds) which are then no longer available to protect the LDL from oxidation. Therefore, it is not an appropriate method to evaluate the protective effect of those substances. Moreover, *ex vivo* determination of LDL resistance to oxidation may not reflect oxidation *in vivo* because the LDL particles are removed from their natural environment and many factors relevant to oxidation of LDL *in vivo* are therefore lacking [22]. Therefore, human intervention studies measuring exclusively *ex vivo* resistance of LDL to oxidation were excluded from the analysis [22,55]. The search resulted in the identification of 22 human intervention studies related to the antioxidant properties of olive polyphenols [26,56–69]. From

those studies, 15 were classified as pertinent and 7 were excluded since they primarily describe the *ex vivo* resistance of LDL to oxidation. The pertinent human studies are summarized in Table I.

Oxidative biomarkers in humans

Plasma levels of oxidized LDL levels

Oxidized low-density lipoproteins (oxLDL) can reflect modification in the lipid and/or protein component of the LDL. These modified LDL particles are proatherogenic, proinflammatory, and highly immunogenic. They play a key role in the development of atherosclerosis and coronary heart diseases [70–72]. A number of studies showed an association between elevated plasma levels of oxLDL and coronary heart disease (CHD) [73–77]. Meisinger *et al.* [78] showed that increased plasma concentrations of oxLDL were predictive of future CHD in apparently healthy men. Recently Holvet *et al.* [79] found that high levels of oxLDL were associated with an increased incidence of metabolic syndrome. The circulating ox-LDL reflects *in vivo* oxidation and is accepted as a marker for coronary heart diseases [73,80]. The olive polyphenols are well known to decrease LDL oxidation *in vitro* and the antioxidant effect was observed *in vivo* in several intervention studies [55].

Plasma oxidized LDL levels were measured in 6 randomized, placebo-controlled, cross-over studies [26,56–58,60,63]. The absolute change can be compared within studies but not between studies since the design of the studies differs widely (time of treatment, doses, study size, etc.). Therefore, mainly the direction of change (increase or decrease) and the significance are reported. Several studies investigated the effects of olive polyphenols on postprandial oxidative stress, which is linked with postprandial lipemia and hyperglycemia. Table II summarizes the results obtained in the human randomized, cross-over, controlled studies on *in vivo* LDL oxidation. The studies consistently showed that plasma levels of oxidized LDL decreased with increased dietary intake of olive polyphenols. In all 6 studies a decrease in plasma oxidized-LDL level was observed in the groups with a higher intake of olive polyphenols compared with those with a low intake of olive polyphenols. Moreover, in 5 of the 6 studies a significant decrease was observed in oxidized-LDL levels between the low- and the high-olive polyphenol groups. The Euroolive study [57], performed in 200 healthy subjects from five European countries, is

Table 1: Tabulated summary of all pertinent human studies and main outcomes

Citation	Intervention	Duration of intake	Population	Test components (daily dosage)	Main outcomes
Gimeno <i>et al.</i> 2007 [56]	Randomized, cross-over, Controlled, double-blind	3 weeks	30 healthy volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (9 mg) Olive oil high phenolic content (20 mg)	Olive polyphenols dose dependently decreased <i>in vivo</i> oxidized LDL and increased resistance of LDL to oxidation and high-density lipoprotein (HDL) cholesterol.
Covas, 2006 (EUROLIPID) [57]	Randomized, cross-over, controlled, double-blind	3 weeks	200 healthy male volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (4 mg) Olive oil high phenolic content (9 mg)	Plasma oxidative stress markers (conjugated dienes, hydroxyl fatty acid, oxidized LDL) and total cholesterol to HDL cholesterol ratio decreased linearly with increasing polyphenol content in olive oil.
Covas, 2006 [58]	Randomized, cross-over, controlled, double-blind	single dose	12 healthy male volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (6 mg) Olive oil high phenolic content (15 mg)	Olive polyphenols dose-dependently decreased <i>in vivo</i> oxidized LDL in the postprandial state.
Salvini, S. 2006 [59]	Randomized, cross-over, double-blind	8 weeks	10 post-menopausal women	Olive oil low phenolic content (7 mg) Olive oil high phenolic content (30 mg)	The high polyphenolic olive oil lowered oxidized DNA damage measured by the comet assay.
Ruano, 2005 [61]	Randomized, cross-over	single dose	21 hypercholesterolemic volunteers	Olive oil low phenolic content (3 mg) Olive oil high phenolic content (16 mg)	Consumption of a meal based on olive oil rich in polyphenolic compounds improved endothelial-dependent vasodilatory response, decreased oxidative stress (lipoperoxides and isoprostanes) and increased the final products of nitric oxide.
Fito, 2005 [60]	Randomized, cross-over, controlled	3 weeks	40 males with stable coronary heart disease	Olive oil low phenolic content (1 mg) Olive oil high phenolic content (8 mg)	Consumption of virgin olive oil rich in polyphenolics decreased <i>in vivo</i> oxidized LDL and lipid peroxide plasma levels and increased glutathione peroxidase activity as compared to refined olive oil consumption. A decrease in systolic blood pressure was also observed with the high phenolic content product.
Leger, 2005 [62]	Open study	4 days	5 males with type I diabetes	Olive phenolic concentrate (first day 25 mg HT and then 12.5 mg HT)	The olive polyphenolic concentrate had no effect on urine isoprostane excretion but significantly decreased serum thromboxane B2 (TXB2) levels.

Table 1: Tabulated summary of all pertinent human studies and main outcomes ()

Citation	Intervention	Duration of intake	Population	Test components (daily dosage)	Main outcomes
Weinbrenner, 2004 [63]	Randomized, cross-over, controlled double-blind	4 days	12 healthy male volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (3 mg) Olive oil high phenolic content (12 mg)	Consumption of olive oil rich in polyphenols decreased plasma oxidized LDL (oxLDL), 8-oxo-dG in mitochondrial DNA, and malondialdehyde in urine and increased HDL cholesterol and glutathione peroxidase activity in a dose-dependent manner, related to the phenolic content of the olive oil administered.
Marrugat, 2004 [26]	Randomized, cross-over, controlled, double-blind	3 weeks	30 healthy volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (2 mg) Olive oil high phenolic content (4 mg)	Olive polyphenols dose-dependently decreased <i>in vivo</i> plasma oxidized LDL and increased <i>ex vivo</i> resistance of LDL to oxidation, HDL cholesterol levels and urinary hydroxytyrosol levels.
Visioli, 2000 [64]	Cross-over	single dose	6 healthy male volunteers	Olive oil + olive phenolic extract (24 mg) Olive oil + olive phenolic extract (49 mg) Olive oil + olive phenolic extract (73 mg) Olive oil + olive phenolic extract (97 mg)	Polyphenolic rich oils dose-dependently decreased urinary isoprostane excretion, a biomarker of <i>in vivo</i> lipid peroxidation processes.
Moschandreas, 2002 [65]	Randomized, cross-over	3 weeks	25 smokers (11 male and 14 females)	Olive oil low phenolic content (3 mg) Olive oil high phenolic content (21 mg)	No change in markers of plasma antioxidant capacity (MDA, FRAP, lipid hydroperoxides) in smokers.
Vissers, 2001 [66]	Randomized, cross-over, controlled	3 weeks	46 healthy volunteers	Refined olive oil content (0 mg phenolic) Virgin olive oil (21 mg phenolic)	<i>Ex vivo</i> resistance of LDL and HDL to oxidation as well as markers of lipid peroxidation were not affected by treatments.
Machowetz, 2007 [67]	Randomized, cross-over, controlled	3 weeks	200 healthy male volunteers	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (4 mg) Olive oil high phenolic content (9 mg)	The phenolic content of the oil had no effect on DNA and RNA oxidation (8-oxo-deoxyguanosine, 8-oxo-guanosine).
Visioli, 2005 [68]	Randomized, cross-over, controlled	7 weeks	21 mildly hyperlipidemic subjects	Refined olive oil (Total HT (free+ esterified) 0 mg) Virgin olive oil (Total HT 7 mg) (a)	Serum TXB2 production decreased and total antioxidant capacity increased after phenolic rich oil intake.

Table I: Tabulated summary of all pertinent human studies and main outcomes ()

Citation	Intervention	Duration of intake	Population	Test components (daily dosage)	Main outcomes
Oubina, 2001 [69]	Cross-over	4 weeks	14 women	High oleic sunflower oil Virgin olive oil (Diet study no fixed amounts of oils)	Total lipid peroxides in serum and TXB2 concentrations in platelet-rich plasma were significantly lower in the virgin olive oil group as compared to the sunflower oil group.

Table II: Effects of olive phenolics on plasma levels of oxidized LDL

Citation	Treatment (daily dosage)	Number of subjects n/N	Change from pre-intervention level	Change from the low phenolic group
Gimeno, 2007 [56]	Low phenolic (0 mg)	30/30	↑ (+3.2 %)	
	Medium phenolic (9 mg)	30/30	↓ (-5.2 %) ^a	↓
	High phenolic (20 mg)	30/30	↓ (-28.2 %) ^a	↓
Covas, 2006a [57]	Low phenolic (0 mg)	182/200	↑ (+2.6 %)	
	Medium phenolic (4 mg)	182/200	↓ (-3 %) ^a	↓ (-5.3 %)
	High phenolic (9 mg)	182/200	↓ (-6.5 %) ^a	↓ (-7.7 %) ^b
Covas, 2006b [58]	Low phenolic (0 mg)	12/12	↑ (+20.9 %) ^a	
	Medium phenolic (6 mg)	12/12	↑ (+8.9 %) ^a	↓
	High phenolic (15 mg)	12/12	↓ (-15.2 %) ^a	↓ ^b
Fito, 2005 [60]	Low phenolic (1 mg)	40/46		
	High phenolic (8 mg)	40/46		↓ (-8 %) ^b
Weinbrenner, 2004 [63]	Low phenolic (0 mg)	12/12	no change	
	Medium phenolic (3 mg)	12/12	↓ (-5 %) ^a	↓ (-16 %)
	High phenolic (12 mg)	12/12	↓ (-35 %) ^a	↓ (-26 %) ^b
Marrugat, 2004 [26]	Low phenolic (0 mg)	30/30	↓ (-18 %)	
	Medium phenolic (2 mg)	30/30	↓ (-12 %)	↑ (+11 %)
	High phenolic (4 mg)	30/30	↓ (-34 %) ^a	↓ (-7 %) ^b

n/N = number of subjects which completed the study / total number of subjects randomized in the study

a = significantly different from the pre-intervention levels ($p < 0.05$)

b = significantly different from the low phenolic group ($p < 0.05$)

the largest clinical study showing that olive polyphenols decreased biomarkers of lipid oxidative damage, such as plasma oxidized-LDL, conjugated dienes, and hydroxyl fatty acids, and provided good evidence for the antioxidant activity of olive polyphenols. The study also confirmed that consumption of olive polyphenols increased plasma hydroxytyrosol levels, showing that olive polyphenols were systemically available. The concentration of phenolic compounds in LDL was directly correlated with the phenolic concentration in the olive oils tested. Moreover, the increase in phenolic content of LDL could account for the increase in resistance of

LDL to oxidation, and the decrease of *in vivo* oxidized LDL, observed in the study.

The doses of olive phenolics showing an effect on plasma oxidized LDL levels ranged from 4 to 20 mg per day; consistent significant effects were observed with doses of about 10 mg per day of total olive phenolic compounds. Hydroxytyrosol or derivatives thereof represent about 50 % of the olive phenolic compounds. The data clearly support the protective effects of olive polyphenols against LDL oxidation.

Table III: Effects of olive phenolics on isoprostane plasma levels

Citation	Treatment (daily dosage)	Number of subjects n/N	Change from pre-intervention level	Change from the low phenolic group
Covas, 2006a [57]	Low phenolic (0 mg)	182/200	↑ (+0.5 %)	
	Medium phenolic (4 mg)	182/200	↓ (-1 %) ^a	↓ (-2.3 %)
	High phenolic (9 mg)	182/200	↑ (+0.2 %) ^a	↓ (-1.5 %)
Covas, 2006b [58]	Low phenolic (0 mg)	12/12	↑ (+29.9 %) ^a	
	Medium phenolic (6 mg)	12/12	↑ (+23.8 %) ^a	↓
	High phenolic (15 mg)	12/12	↑ (+16.5 %) ^a	↓
Ruano, 2005 [61]	Low phenolic (3 mg)	21/21	↑ (+25 %)	
	High phenolic (16 mg)	21/21	↓ (-30 %) ^a	↓ ^b
Leger, 2005 [62]	HT (12.5 mg)	5/5	no effect	no control group
Weinbrenner, 2004 [63]	Low phenolic (0 mg)	12/12	no effect	
	Medium phenolic (3 mg)	12/12	no effect	no effect
	High phenolic (12 mg)	12/12	no effect	no effect
Visioli, 2000 [64]	Phenolic extract (24 mg)	6/6	not reported	
	Phenolic extract (49 mg)	6/6	not reported	↓ (-16 %)
	Phenolic extract (73 mg)	6/6	not reported	↓ (-34 %)
	Phenolic extract (97 mg)	6/6	not reported	↓ (-32 %)
Visioli, 2005 [68]	Total HT (0 mg)	22/22	↑	
	Total HT (7 mg)	22/22	↓ ^a	↓

n/N = number of subjects which completed the study / total number of subjects randomized in the study

a = significantly different from the pre-intervention levels ($p < 0.05$)

b = significantly different from the low phenolic group ($p < 0.05$)

Isoprostane levels in plasma or urine

F2-isoprostanes are produced by nonenzymatic, free-radical-catalyzed peroxidation of arachidonic acid. They are considered as markers of lipid peroxidation and can also exert potent biological actions. They can be quantified in human body fluids such as plasma and urine. Table III summarizes the results obtained in clinical intervention studies on plasma isoprostane levels after consumption of olive polyphenols.

In several studies, a trend toward a decrease in plasma isoprostane levels was observed with increasing intake of olive polyphenols [57,58,64,68]. The olive polyphenols also tended to decrease the postprandial rise in plasma isoprostane levels [58]. However, a significant decrease in plasma isoprostane levels between the groups receiving a low phenolic diet and a high phenolic diet was only observed in one study from Ruano *et al.* [61]. No effect was observed by Weinbrenner *et al.* [63] in a small study. In another small open study Leger *et al.* [62] reported that an olive polyphenolic extract had no effect on urinary

isoprostane levels but significantly lowered serum thromboxane levels. Those data were confirmed by Visioli *et al.* in an unpublished study cited in [81]. Overall, the studies suggest that a modest effect on plasma isoprostane levels may be observed after ingestion of olive polyphenols. However, in 4 of 7 studies, only 5 to 12 subjects were studied. Thus, further studies are needed to clarify the effect of olive polyphenols on this marker of oxidative stress, which seems to be less sensitive to olive polyphenols than LDL oxidation.

DNA damage

Cellular DNA damage can be induced by reactive oxygen species and different techniques have been developed to measure DNA damage by quantifying the oxidation of nucleotides. Oxidatively-modified nucleobases (8-oxo-deoxyguanosine (8-oxo-dG), 8-oxo-guanosine, 8-oxo-guanine) can be measured in plasma and urine. However, these oxidatively modified nucleobases can also arise from degradation of

Table IV: Effects of olive phenolics on various oxidative biomarkers

Citation	Test components (daily dosage)	Change low phenolic vs. high phenolic
Covas, 2006 [57]	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (4 mg) Olive oil high phenolic content (9 mg)	↓ Conjugated dienes ^a ↓ Hydroxy fatty acids ^a ↓ total cholesterol ^a ↓ HDL cholesterol ^a
Covas, 2006 [58]	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (6 mg) Olive oil high phenolic content (15 mg)	↑ ox-LDL antibodies ^a
Salvini, S. 2006 [59]	Olive oil low phenolic content (7 mg) Olive oil high phenolic content (30 mg)	↑ Plasma antioxidant capacity ↓ oxidized DNA bases (comet assay)
Ruano, 2005 [61]	Olive oil low phenolic content (3 mg) Olive oil high phenolic content (16 mg)	↓ lipoperoxides (LPO) ^a ↑ ischemic reactive hyperemia
Fito, 2005 [60]	Olive oil low phenolic content (1 mg) Olive oil high phenolic content (8 mg)	↑ ox-LDL antibodies ↓ lipoperoxides ^a ↑ glutathione peroxidase ^a ↓ systolic blood pressure ^a
Weinbrenner, 2004 [63]	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (3 mg) Olive oil high phenolic content (12 mg)	↓ MDA in urine ^a ↓ DNA damage (8 oxo-deoxyguanosine in urine) ^a ↑ glutathione peroxidase activity ^a ↑ glutathione reductase activity ^a ↑ HDL-cholesterol ^a
Marrugat, 2004 [26]	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (2 mg) Olive oil high phenolic content (4 mg)	↑ ox-LDL antibodies
Moschandreas, 2002 [65]	Olive oil low phenolic content (3 mg) Olive oil high phenolic content (21 mg)	FRAP (no effect) MDA (no effect) Protein carbonyls (no effect) ↓ Lipid hydroperoxides
Vissers, 2001 [66]	Refined olive oil (0 mg phenolic) Virgin olive oil (21 mg phenolic)	FRAP (no effect) MDA (no effect) Lipid hydroperoxides (no effect) Protein carbonyls (no effect)
Machowetz, 2007 [67]	Olive oil low phenolic content (0 mg) Olive oil medium phenolic content (4 mg) Olive oil high phenolic content (9 mg)	DNA, RNA damage (no effect)
Visioli, 2005 [68]	Refined olive oil (Total HT (free+ esterified) 0 mg) Virgin olive oil (Total HT 7 mg) (a)	↑ Plasma antioxidant capacity ^a ↓ TXB2 level in serum ^a
Oubina, 2001 [69]	High oleic sunflower oil Virgin olive oil (Diet study no fixed amounts of oils)	↓ TXB2 in stimulated platelets ^a TXB2, 6-keto-PGF1 α in urine (no effect) Lipid peroxides (no effect)

MDA: Malondialdehyde; FRAP: ferric reducing ability of plasma; TXB2: Thromboxane B2
a significant different from the low phenolic group (p<0.05)

oxidized nucleotides in the DNA precursor pool and not just from removal of oxidized nucleotide residues from DNA by repair processes. Therefore, the concentrations of oxidized nucleotides in plasma or urine may not truly reflect rates of oxidative damage to DNA. Moreover, those measurements in human body fluids (plasma, urine) are difficult to carry out

reliably and the best method for measuring DNA oxidative damage still needs to be defined [82,83]. The effects of olive polyphenols on DNA damage was reported in three human intervention studies [59,63,67]. Weinbrenner *et al.* showed that olive polyphenols significantly decreased 8-oxo-dG in urine and plasma of healthy volunteers consuming a

very low antioxidant diet [63]. Salvini *et al.* reported a protective effect of olive polyphenols on DNA oxidation, measured by the COMET assay, in postmenopausal women [59]. However, Machowetz *et al.* [67] found no significant effect of the olive polyphenols on DNA oxidation in subjects from the larger EUROLIPID study. Thus, the effects of olive polyphenols on DNA oxidative damage needs further well-controlled human studies and more sensitive and better methods are needed to detect small differences after dietary treatment with olive polyphenols.

Other oxidative biomarkers

The effects of olive polyphenols on additional oxidative biomarkers have been described in several studies and are summarized in Table IV.

Lipid peroxidation (lipoperoxides, lipid hydroperoxides) was significantly decreased in three studies [57,60,61] and tended to decrease in one study [65] while no effect was observed in two studies [66,69]. Two studies reported that the plasma antioxidant capacity increased after ingestion of olive oil rich in polyphenols, but not after low polyphenolic olive oil, indicating that olive polyphenols decrease lipid oxidation [59,68]. In three studies the plasma levels of oxidized LDL antibodies were increased [26,58,60]. Oxidized LDL particles are immunogenic and induce the production of autoantibodies. The role of autoantibodies to oxLDL in cardiovascular diseases is controversial. However, in healthy subjects, an inverse association between autoantibodies against oxLDL and carotid artery intima-media thickness or oxLDL was reported [84,85]. Moreover, Miller *et al.* [86] showed that a diet that reduced oxidative stress also increased antibodies to oxLDL. Thus, an increase in oxidized LDL antibodies may be atheroprotective as a result of a decreased oxidative stress. Malondialdehyde was significantly decreased in one study where the participants consumed a very low antioxidant diet [63]. In two studies no effect was observed [65,66]. However, in one of the studies the low phenolic groups already consumed 3 mg of olive phenolics and the study subjects were smokers [65]. The negative impact of smoking on antioxidant capacity probably outweighed the benefit of olive phenolics in this study. The study of Weinbrenner *et al.* showed a significant effect on all the measured biomarkers of oxidation on the background of a very low phenolic diet [63]. This study pointed out one of the problems in assessing the antioxidant effects of phenolics, since the human body is normally exposed

to antioxidant phenolics which are present in the background diet. It should be noted that in several studies graded amounts of olive phenolics were tested, making it difficult to show significant differences when compared with a control group consuming olive phenolics in the background diet. This could explain some of the inconsistent effects observed between several human studies. Moreover, the studies done in a postprandial state suggest that the antioxidant effects of olive polyphenols is more likely to be observed under oxidative stress conditions [58]. Finally, two studies reported a significant increase in the glutathione peroxidase activity [60,63]. Glutathione peroxidase is an endogenous antioxidant enzyme that protects organisms from oxidative damage by neutralizing free reactive oxygen species within the cells. Thus, olive polyphenols have indirect antioxidant effects by activating such endogenous defense systems. Collectively the data suggest that olive polyphenols decrease various markers of oxidative stress, particularly markers of lipid peroxidation.

Conclusion

Human intervention studies showed that olive phenolics decrease plasma levels of oxidized-LDL after dietary intakes of olive phenolics ranging from 4 to 20 mg with consistent significant effects being observed with doses of about 10 mg of total olive phenolic compounds. The effects of olive polyphenols on DNA oxidative damage are less consistent and need further, well-controlled human studies. The human studies also indicate that olive polyphenols improve several markers of oxidative damage, such as plasma levels of lipid hydroperoxides, malondialdehyde, and plasma antioxidant capacity. An increase in glutathione peroxidase activity was also observed suggesting that olive polyphenols could stimulate the body's own antioxidant defense systems. In conclusion, olive polyphenolics have antioxidant activities in humans. However, further larger human studies are needed to confirm the effects of olive polyphenolics on biomarkers of oxidative stress.

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