Bioavailability and antioxidant effects of olive oil phenolic compounds in humans: a review

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Summary. Olive oil, the main source of fat in the Mediterranean diet, is a functional food which besides having a high level of monounsaturated fatty acid contains several minor components with biological properties. For some olive oil minor components, such as the antioxidant phenolic compounds, a large body of studies, mainly experimental or in animal models, have been performed. Randomized, controlled, clinical trials in humans are required to provide evidence that olive phenolic compounds contribute significantly to health benefits in order to give recommendations at population level. Here, we summarize the state of the art of the body of knowledge, and to which extent we have evidence, of the bioavailability and of the antioxidant benefits of olive oil phenolic compounds in humans.

Key words: olive oil, phenols, bioavailabilty, hydroxytyrosol, tyrosol.

Riassunto *(Biodisponibilità di composti fenolici dell'olio di oliva e loro effetti antiossidanti nell'uomo)*. L'olio di oliva, la principale fonte di grasso nella dieta mediterranea, è un cibo funzionale che contiene, oltre ad elevate concentrazioni di acidi grassi monoinsaturi, diversi componenti minori con spiccate attività biologiche. Per alcuni di questi componenti minori, come i composti fenolici antiossidanti, sono stati condotti molti studi soprattutto *in vitro* e su modelli animali. Per offrire evidenze scientifiche certe sui benefici che i composti fenolici dell'oliva possono esercitare sulla salute umana, è necessario condurre trial clinici, randomizzati e controllati, sull'uomo, così che sia possibile trasferire i dati scientifici in raccomandazioni nutrizionali a livello di popolazione. Questa rassegna vuole riassumere lo stato dell'arte delle conoscenze, ed evidenziarne il livello di certezza scientifica, sulla biodisponibiltà dei composti fenolici dell'olio di oliva e sui benefici antiossidanti che essi esercitano nell'uomo.

Parole chiave: olio di oliva, biodisponibilità, fenoli, idrossitirosolo, tirosolo.

INTRODUCTION

Olive oil, the main source of fat in the Mediterranean diet [1], is a functional food which besides having high amounts of monounsaturated fatty acid (MUFA) contains minor components with biological properties. The content of minor components of an olive oil varies, depending on the cultivar, climate, ripeness of the olives at harvesting, and the processing system for the type of olive oil: virgin, common (ordinary), or pomace [2]. Virgin olive oil is produced by direct press or centrifugation methods. Virgin olive oils with an acidity greater than 3.3 degrees are submitted to a refination process in which some components, mainly phenolic compounds and to a lesser degree squalene, are lost [3]. By mixing virgin and refined olive oil a common olive oil (olive oil, UE 1991) is marketed. After virgin olive oil production the rest of the olive drupe and seed is processed and submitted to a refination process, and pomace olive oil, to which a certain quantity of virgin olive oil is added, is marketed. Minor components of virgin olive oil are classified in the unsaponificable compounds (squalene, sitosterols, triterpenes, pigments, etc.), defined as the fraction extracted with solvents after the saponification of the oil [4], and the soluble ones which includes the phenolic compounds.

Olive oil phenolic compounds are the most well studied and characterized minor olive oil components. The major phenolic compounds in olive oil are: simple phenols (*i.e.*, hydroxytyrosol, tyrosol); polyphe-

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nols (oleuropein glucoside); secoiridoids (SID), the dialdehydic form of oleuropein (SID-1) and ligstroside (SID-2) lacking a carboxymethyl group, and the aglycone form of oleuropein glucoside (SID-3) and ligstroside (SID-4); and lignans, *i.e.*, (+)-pinoresinol and (+)-1-acetoxypinoresinol (3). Tyrosol, hydroxytyrosol and their secoiridoids derivatives represents around 30%, and other conjugated forms such as oleuropeine and ligstroside aglycone represents almost half, of the total phenolic content of a virgin olive oil. Around 80% or more of the olive oil phenolic compounds are lost in the refination process, thus, their content is higher in virgin olive oil (around 230 mg/ kg, common range 130-350 mg/ kg) than in other olive oils [3].

In *in vitro* and *ex vivo* models, olive oil phenolics have shown to have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation [3, 5-7]. They are also able to prevent the endothelial dysfunction by decreasing the expression of cell adhesion molecules [8], and increasing nitric oxide (NO) production and inducible NO synthesis [9] by quenching vascular endothelium intracellular free radicals [10]. Also, olive oil phenolic compounds inhibited platelet-induced aggregation [11] and have been reported to enhance the mRNA transcription of the antioxidant enzyme glutathione peroxidase [6]. This last issue, however, seems to be dependent on the tissue in which the gene expression was evaluated [6, 12]. Other potential activities include anti-inflammatory and chemopreventive activity [13, 14]. In animal models, olive oil phenolics retained their antioxidant properties *in vivo* [15, 16] and delayed the progression of the atherosclerosis [17].

So far, most of the cardio-protective effect of olive oil in the context of the Mediterranean diet has been attributed to its high MUFA content. Recently, the Federal Drug Administration (FDA) of the USA permitted a claim on olive oil labels concerning: "the benefits on the risk of coronary heart disease (CHD) of eating about 2 tablespoons (23 grams) of olive oil daily, due to the monounsaturated fat (MUFA) in olive oil" [18]. It must be noticed, however, that oleic acid is one of the predominant fatty acids in widely-consumed animal foods in Western diets, such as poultry and pork [19]. A direct association of meat intake with the plasma oleic acid concentration was observed in a Swedish female population [20]. In this population, oleic acid plasma concentrations were higher than those of females of Granada in Spain, without differences in polyunsaturated (PUFA) levels [20]. Thus, perhaps a high oleic acid intake is not the sole primary responsible agent for the healthy properties of olive oil. In spite of the promising role for health displayed in experimental studies, evidence of the benefits of olive oil phenolic compounds consumption in humans is still on the debate. If the beneficial effect of olive oil in humans can be attributed solely to is MUFA content, any type of olive oil, rapeseed/canola oil, or MUFA-enriched fat would provide the same health benefits. Thus, public health implications are involved in order to specifically recommend olive oil, and which type of olive oil, (*i.e.*, virgin olive oil rich in phenolic compounds) as individualized nutritional strategies for CHD prevention. On the basis of the Evidence-Based Medicine, adequate scientific evidence, is required before to formulate nutritional recommendations to the population. The scientific evidence required is that provided by randomized, controlled, human clinical trials (level I of Evidence) and to some extent by large cohort studies (level II of Evidence). Of course, the level of evidence of a particular study depends, not only on the design, but also on the quality of the study (external and internal validity, homogeneity of the sample and statistical power). Finally, evidence emerges from the agreement of the results among several similar studies [21, 22]. Here, we will focus in the antioxidant properties of olive oil phenolic compounds in humans, the state of the art of the body on knowledge, and to which extent we have scientific evidence on that issue.

BIOAVAILABILITY OF OLIVE OIL PHENOLIC COMPOUNDS

On the basis of the scavenger capacity of phenolic compounds on free radicals generated by the faecal matrix [14] and those induced in the intestinal epithelium cells [23], it has been proposed that non absorbable phenolic compounds can exert local antioxidant activities in the gastrointestinal tract [24]. However, one of the prerequisites to assess the *in vivo* olive oil phenolic compounds physiological significance is to determine their bioavailability in humans. Tyrosol and hydroxytyrosol, the major olive oil phenolic compounds present in olive oil as simple forms or conjugates [3], rise early after virgin olive oil ingestion reaching a peak at around 1 h in plasma [25, 26] and 0-2 h in urine [25, 27, 28]. In an elegant approach Vissers *et al.* [29] showed oleuropein to be absorbed in the small intestine of ileostomy patients, metabolized in the body, and recovered in urine as hydroxytyrosol. Tyrosol and hydroxytyrosol and their derivatives are absorbed by humans in a dose-dependent manner with the phenolic content of the olive oil administered [30]. Even from moderate doses (25 mL (22 g/day) [26, 31, 32] lower than those reported as usual in the Mediterranean areas (30-50 g/day) [33]. The dose-dependent increase of tyrosol and hydroxytyrosol with the phenolic content of the olive oil has been observed, both in plasma and urine, after a single dose [26, 30, 31], short- [31] and long-term [32, 34] consumption of real-life doses of similar olive oils, but with differences in their phenolic content. Due to this, urinary tyrosol and hydroxytyrosol can be considered as biomarkers of phenolic compounds from olive oil consumption, and an useful tool for monitoring compliance in clinical intervention studies. Concerning the dose-response relationship, urinary

concentrations of tyrosol were dependent on the administered tyrosol dose, whereas hydroxytyrosol urinary concentrations tended to accumulate [35]. The endogenous production of hydroxytyrosol, as a metabolite of the dopaminergic pathway, could account for this fact. In fact, homovanillic acid, one of the main metabolites of dopamine, has also been reported as a major metabolite of hydroxytyrosol [36]. Around 98% of tyrosol and hydroxytyrosol are present in plasma and urine in conjugated forms, mainly glucuronoconjugates, suggesting an extensive first pass intestinal/hepatic metabolism of the ingested primary forms [25, 28]. Due to this, olive oil phenolics bioactivity it is likely to be derived mainly from its biological metabolites. In fact, some preliminary reports support the view that the 3-O-glucuronide of hydroxytyrosol shows stronger activity as a radical scavenger than hydroxytyrosol itself [37]. From data obtained after plasma enzymatic and acidic hydrolysis, hydroxytyrosol and its 3-O-methylated biological metabolite are present in plasma as around 65% as glucuronate and 35% in other conjugated, such as sulphate, forms. *Table 1* shows the plasma pharmacokinetic parameters for both phenolic compounds after virgin olive oil ingestion.

ANTIOXIDANT EFFECT OF OLIVE OIL PHENOLIC COMPOUNDS IN HUMANS

Several randomized, cross-over, controlled, human studies, which potentially could provide first level of evidence on the *in vivo* antioxidant effect of sustained doses of phenolic compounds from olive oil have been performed. Extensive differences among these studies exits in: the experimental design, control of diet, sample population, age of the participants, measurement or not of markers of the compliance of the intervention, and in the sensitivity and specificity of the oxidative stress biomarkers evaluated. In four studies performed until year 2001 with healthy volunteers, there was no evidence that the consumption of phenols, in the amounts provided by dietary olive oil, accounted for benefits neither on the *in vitro* susceptibility of LDL against oxidation [38] nor in other oxidative markers such as plasma malondialdehyde, lipid hydroperoxides, or protein carbonyls [39, 40]. In contrast, in more recent years, protective effects of olive oil phenols on *in vivo* circulating oxidized LDL and DNA oxidation, but not in plasma F2-isoprostanes, were found in healthy male subjects [31, 32]. Differential characteristics of these studies, in comparison with the previous referred to above, were subjects submitted to a strict very low-antioxidant diet during washout and intervention periods [31], or to a controlled diet in order to avoid high antioxidant consumption [32]. In these studies [31, 32], low phenolic olive oil was used for cooking purposes during intervention periods, and for raw and cooking purposes during washout periods. This fact permitted the homogenization of both the main fat ingestion of participants and the LDL fatty acid content. The type of fat ingested influences the oxidative damage to lipids [41]. Differences among participants in the fat ingested, both for raw and cooking purposes during washout periods and for cooking purposes during intervention periods, can be an important confounder in the assessment of the antioxidant effects of the phenolic compounds from olive oil. In addition, urinary tyrosol and hydroxytyrosol were assessed as biomarkers of the compliance of the interventions [31, 32].

When the antioxidant effect of olive oil phenolic compounds was tested in patients in which an enhanced oxidative stress status has been reported [42-44], the pattern obtained was homogeneous as overall. A protective effect of virgin olive oil, versus other olive oils, on the resistance of LDL to oxidation was found in studies involving peripheral vascular disease [45] or hyperlipidemic patients [46]. In mildly hyperlipidemic patients an increase in the total antioxidant capacity, without changes in plasma F2-isoprostanes, related with the phenolics from the olive oil consumed has also been reported [47]. Protective effects related with the phenolic content of the olive oil on circulating oxidized LDL and lipid peroxides in stable CHD patients [34], and on DNA oxidation in postmenopausal women [48] ha-

Table 1 | *Plasma pharmacokinetic parameters for hydroxytyrosol (HT) and 3-O-methyl-HT after ingestion of 40 mL of virgin olive oil with a phenolic content of 366 mg/kg olive oil*

Hydrolysis	C_{max} (µg/L)	t_{max} (h)	t ½ (h)	AUC_{0-8h}
Acidic				
HT	25.83 (12.96)	0.58 (0.26)	3.12 (1.5)	72 (26)
3-O-methyl-HT	3.94 (2.13)	0.88 (0.54)	2.96 (0.9)	12 (4)
Enzymatic				
HT	17.09 (6.84)	0.54 (0.21)	3.01 (1.1)	47 (12)
3-O-methyl-HT	3.02 (1.53)	0.82 (0.53)	2.37 (1.3)	10 (2.9)

Values are expressed as mean (SD).

C_{max}: maximal plasma concentration of compound; t_{max}: time taken to reach C_{max}; t ½: half-life of elimination; AUC_{0-8h}: area under the curve from 0 to 8 hours. Adapted from: Miró-Casas et al., Clin Chem 2003 [25].

ve been recently described. *Table 2* summarizes the randomized, crossover, controlled studies on the sustained effect of phenolic compounds from olive oil on lipids and DNA oxidative damage in individuals with enhanced oxidative stress.

On the basis of the studies referred above, conclusions of the Consensus Report, made by the Expert Panel of the International Conference of Olive Oil and Health, held in Jaen, Spain October 2004 [49, 50], on the benefits of minor olive oil components in humans, concluded: 1) data regarding the benefits of olive oil phenolic compounds in humans from real-life daily doses of olive oil are still controversial; 2) the protective effects on lipid oxidation, in the human trials performed, being better displayed in oxidative stress conditions; 3) the best results obtained on lipid oxidation parameters were displayed in those markers directly associated with LDL oxidation; and 4) carefully controlled studies in appropriate populations (individuals with high oxidative status), or with a large sample size (in the case of healthy individuals), are required to definitively establish in which conditions phenolics from olive oil can exert their most beneficial effect controlling oxidative stress.

Concerning the fact that the protective effects of olive oil phenolic compounds on lipid oxidation are best displayed in oxidative stress conditions, this could be linked to the fact that the balance of prooxidant and antioxidant reactions is well regulated in the body. Due to this, an intervention with an antioxidant-rich compound without any oxidative stress involved may exert only a marginal effect. In this sense, and after a single dose of three similar olive oils, but with low, medium, and high phenolic content, neither hypertryglyceridemia nor oxidative stress were observed in any case after a 25 mL dose [26], whereas the opposite was observed after a 40 mL dose [51]. In this last situation, however, the degree of oxidative stress was modulated, in an inverse relationship, by the phenolic content of the olive oil [51]. Concerning the statement that a large sample size of healthy individuals would be required to observe benefits on the oxidative biomarkers linked with the phenolic content of the olive oil, the recent results of the EUROLIVE study have confirmed this hypothesis [52, 53]. The EUROLIVE (The effect of olive oil consumption on oxidative damage in European populations) study was a large, crossover, multicentre, clinical trial performed in 200 individuals from 5 Europeans countries. Participants were randomly assigned for receiving 25 mL/day of three similar olive oils, but with differences in their phenolic content, in intervention periods of 3 weeks preceded by two-week washout periods. All olive oils increased HDL-cholesterol and the ratio between

Table 2 | *Randomized, crossover, controlled studies on the sustained effect of phenolic compounds from olive oil on lipids and DNA oxidative damage in individuals with enhanced oxidative stress*

Subjects	Intervention period	Intervention period	Washout	Oxidative markers	Effects	Reference
24 (men) Peripheral Vascular disease	Virgin *vs* refined all purposes	3 months	3 months without olive oil	Lipid peroxides in LDL Macrophage plasma oxidized LDL uptake	Decrease with olive oil phenol content (all markers)	Ramírez-Tortosa *et al.* (1999) [45]
12 healthy men submitted to a very-low antioxidant diet	High *vs* Medium *vs* Low phenol olive oil (25 mL/d, raw)	4 days: refined olive oil for cooking; very low antioxidant diet	10 days: refined olive oil for all purposes; very low antioxidant diet	Plasma oxidized LDL MDA in urine 8-oxo-dG in urine and lymphocytes F_2-isoprostanes GSH-Px	Decrease with olive oil phenol content (all markers) None Increase with olive oil phenol content	Weinbrenner *et al.* (2004) [31]
22 hiperlipemic patients (12 men 10 women)	Virgin *vs* refined (raw) (40 mL/day)	7 weeks usual diet	4 weeks with usual diet	Plasma antioxidant capacity F_2-isoprostanes	Increase with olive oil phenol content None	Visioli *et al.* (2005) [47]
Coronary heart disease patients (40 men)	Virgin *vs* refined (raw) (50 mL/day)	3 weeks with refined olive oil for cooking	2 weeks with refined olive oil for all purposes	Plasma oxidized LDL and Lipid peroxides GSH-Px	Decrease with olive oil phenol content Increase with olive oil phenol content	Fitó *et al.* (2005) [34]
10 women Post-menopausal	High *vs* Low phenol virgin olive oil	8 weeks	2 weeks	Comet assay	Decrease in DNA oxidative damage olive oil phenol content	Salvini *et al.* (2006) [48]

MDA: malondialdehyde; 8-oxo-dG: 8-oxo-deoxyguanosine; GSH-Px: glutathione peroxidase; DNA: deoxyribonucleic acid.

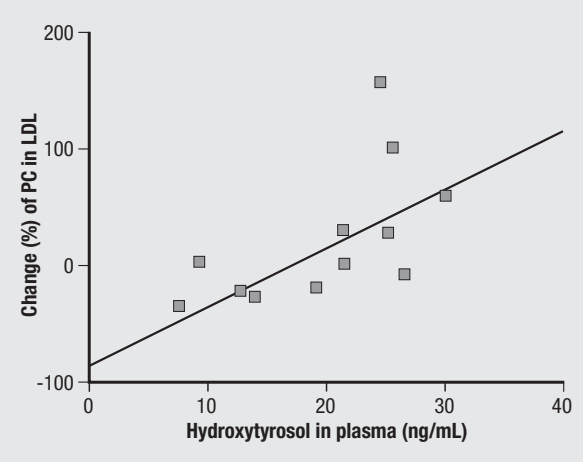

Fig. 1 | *Relationship between the change in the total phenolic content (PC) of the LDL and plasma hydroxytyrosol concentrations at 30 minutes after ingestion of 40 mL of a high phenolic content (366 mg/kg) olive oil. R = 0.780, P = 0.009, Spearman's correlation coefficient. Adapted from: Covas et al., Free Radic Biol Med, 2006 [51].*

reduced and oxidized forms of glutathione, and decreased triglycerides, total/HDL cholesterol ratio, and DNA oxidative damage. Consumption of medium- and high-phenolic content olive oil decreased LDL/HDL cholesterol ratio, oxidized LDL, conjugated dienes, and hydroxy fatty acids. The greatest effects on increasing HDL cholesterol levels and decreasing lipid oxidative damage were observed after the high phenolic olive oil consumption.

The fact that in general the role of olive oil phenolic compounds on oxidative damage obtained were displayed in those markers directly associated with LDL oxidation, both in healthy individuals and in oxidative stress conditions, could be explained by the increase in the antioxidant content of the LDL observed after virgin olive oil ingestion [54]. The susceptibility of LDL to oxidation depends not only on its fatty content, but also on the LDL content of antioxidants (*e.g.*, vitamin E and polyphenols) [55]. In experimental [56], as well as in *in vivo* human studies [51], phenolic compounds bound to human LDL increased in a dose dependent manner with the phenolic content of the olive oil administered *(Figure 1)*. Very recently, the capacity of olive oil phenolic compounds, and its metabolites, to bind the LDL lipoprotein has been reported [57]. Phenolic compounds which can bind LDL are likely to perform their peroxyl scavenging activity in the arterial intima, where full LDL oxidation occurs in microdomains sequestered from the richness of antioxidants present in plasma [58].

In summary, olive oil phenolic compounds are bioavailable in humans, can increase the antioxidant content of the LDL lipoprotein, and exert *in vivo* antioxidant properties. Although the clinical significance of the changes in oxidative damage to lipids associated to the presence of a high phenolic content in the olive oil are, at present, unknown the combined effect of the MUFA and the phenolic content of virgin olive oil could reduce the oxidative lipid damage, particularly in oxidative stress conditions. Further studies are required to evaluate the effect of olive oil phenolic compounds on DNA oxidative damage.

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