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

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Objective: We reviewed the bioavailability and antioxidant effects of phenols from extra virgin olive oil.
Search strategy: We searched the MEDLINE database for the years 1966–2002. To review the bioavailability of olive oil phenols, we selected animal and human studies that studied the absorption, metabolism, and urinary excretion of olive oil phenols. We also estimated the intake of the various phenols in the Mediterranean area. To review the antioxidant effects of olive oil phenols, we included human and animal studies on the effect of olive oil phenols on markers of oxidative processes in the body. We excluded studies without a proper control treatment and studies in which the antioxidant effects of phenols could not be disentangled from those of the fatty acid composition of olive oil.
Results: Bioavailability studies in humans show that the absorption of olive oil phenols is probably larger than 55–66 mol%, and that at least 5% is excreted in urine as tyrosol and hydroxytyrosol. Animal studies suggest that phenol-rich olive oil lowers oxidisability of *ex vivo* low-density lipoprotein (LDL) particles or lowers markers in urine of oxidative processes in the body. In five out of seven human studies, however, these effects of phenols were not found. There are no data on the phenol concentrations in plasma that are attainable by intake of olive oil. We estimated that 50 g of olive oil per day provides about 2 mg or $\sim$13 μmol of hydroxytyrosol-equivalents per day, and that the plasma concentration of olive oil phenols with antioxidant potential resulting from such an intake can be at most 0.06 μmol/l. This is much lower than the minimum concentrations of these phenols (50–100 μmol) required to show antioxidant activity *in vitro*.
Conclusion: Although phenols from olive oil seem to be well absorbed, the content of olive oil phenols with antioxidant potential in the Mediterranean diet is probably too low to produce a measurable effect on LDL oxidisability or other oxidation markers in humans. The available evidence does not suggest that consumption of phenols in the amounts provided by dietary olive oil will protect LDL against oxidative modification to any important extent.
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Keywords: olive oil; phenols; antioxidants; LDL oxidation; bioavailability; absorption; human; animal

Introduction

Oxidation of low-density lipoproteins (LDL) is hypothesised to play an important role in the development of atherosclerosis, an underlying factor of cardiovascular diseases. LDL oxidation might be prevented or reduced by intake of antioxidants such as vitamin E. Other compounds with potential antioxidant effects are dietary phenols. Phenols are compounds with an aromatic ring structure with one or more hydroxyl groups. Phenols with two or more hydroxyl groups show antioxidant capacity *in vitro*, whereas phenols with one hydroxyl group have little or none (Rice-Evans *et al*, 1996; Leenen *et al*, 2002). Extra virgin olive oil contains phenols with either one or two hydroxyl groups (Figure 1). Today olive oil is marketed as being healthier than other vegetable oils because of the presence of these phenols, but a pertinent question is whether this suggestion or claim is correct.

This article reviews the evidences from human and animal studies on the potential of olive oil phenols to protect LDL against oxidation. Three important questions in this context are: (1) can olive oil phenols affect oxidative processes in the human body?; (2) how much of the phenolic compounds from olive oil are absorbed in the human body?; and (3) how are they subsequently metabolised? We first describe the

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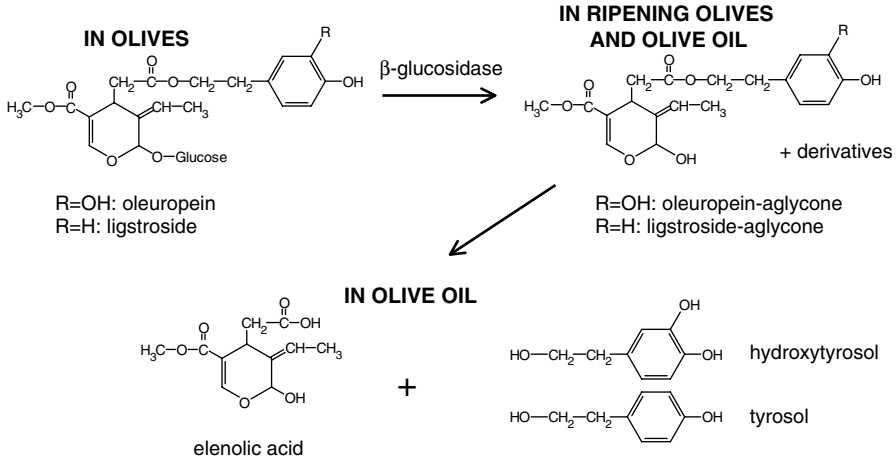

Figure 1 Structures of phenols present in olives and olive oil, their degradation into aglycones during ripening, and hydrolysis of aglycones into tyrosol and hydroxytyrosol.

possible effects of olive oil on coronary heart disease, the chemistry of the olive oil phenols, and their estimated intake.

Olive oil and the risk of coronary heart disease

Keys and co-workers showed that in the period between 1960 and 1975 men in Southern European countries such as Italy, Greece, and Yugoslavia had a much lower incidence of coronary heart disease than men in Northern Europe. These differences between countries could be largely explained by differences in the ratio of monounsaturated to saturated fatty acids in the diet (Keys *et al*, 1986). This would suggest that in particular the type of dietary fat in the Mediterranean area, mainly olive oil, protects against coronary heart disease, because olive oil has a high ratio of monounsaturated to saturated fatty acids.

Controlled dietary trials in humans have shown that replacement of dietary saturated fatty acids with monounsaturated oleic acid (C18:1n-9) from olive oil decreases plasma LDL concentrations, which presumably contributes to the low incidence of coronary heart diseases (Katan *et al*, 1995). It has also been suggested that a high-monounsaturated fat diet lowers the risk of coronary heart disease by producing LDL particles that are enriched in oleic acid at the expense of linoleic acid (C18:2n-6). Such a change in fatty acid composition renders LDL particles more resistant to oxidative modification (Reaven *et al*, 1991; Berry *et al*, 1992; Bonanome *et al*, 1992; Mata *et al*, 1997).

Oleic acid, however, may not be the only component of olive oil protecting LDL from oxidation; in particular, the phenols in extra virgin olive oil could be effective antioxidants. The oxidative modification hypothesis of atherosclerosis states that LDL particles are oxidatively modified and then taken up by macrophages inside the arterial wall. Dietary antioxidants might inhibit atherogenesis by inhibiting oxidation of LDL and accumulation of LDL in macrophages (Witztum & Steinberg, 1991). When olive oil is ingested, the phenols might dissolve into or attach to LDL particles in plasma, where they may prevent LDL from oxidation.

Chemistry and content of phenols in olive oil

The types of phenols in extra virgin olive oil are different from those of the olive fruit. The olives mainly contain the polar glycosides oleuropein and ligstroside. Oleuropein is the ester of elenolic acid with 3,4′-dihydroxyphenylethanol (hydroxytyrosol), and ligstroside is the ester of elenolic acid with 4-hydroxyphenylethanol (tyrosol). Oleuropein and ligstroside are the parent compounds of the less polar oleuropein- and ligstroside-aglycones. Oleuropein- and ligstroside-aglycones are formed by removal of the glucose moiety from the oleuropein- and ligstroside-glycoside by β-glucosidase during ripening. Those aglycones and their various derivatives are the most abundant phenols in olive oil. The derivatives differ mainly in their ring structure (Montedoro *et al*, 1993; Owen *et al*, 2000), which can either be open or closed in two different forms (unpublished data, personal communication from Dr S van Boom). The polar compounds hydroxytyrosol and tyrosol are the end products of hydrolysis of oleuropein- and ligstroside-aglycones or their derivatives in olives and olive oil (Figure 1).

The concentration of phenols in extra virgin olive oil varies from 50 to 800 mg/kg (Visioli & Galli, 1995), with a mean value for commercial olive oil of approximately 180 mg/kg (Owen *et al*, 2000; unpublished data, personal communication from Dr S van Boom). The phenol concentration in olive oil depends on variety, climate, area of growth, latitude, and ripeness of the olive. The phenols, and in particular the *ortho*-diphenols, have been demonstrated to contribute considerably to the oxidation stability of the oil (Gutfinger, 1981; Papadopoulos & Boskou, 1991; Rice-Evans *et al*, 1996; Visioli & Galli, 1998). *Ortho*-diphenols are the phenols with two adjacent hydroxyl groups to the ring structure: hydroxytyrosol and oleuropein and its derivatives (Figure 1).

Intake of phenols from olive oil

Intake of olive oil in the Mediterranean countries is estimated to be 30–50 g/day, based on the *per capita* disappearance of 10–20 kg of olive oil per year in Greece, Italy, and Spain (Helsing, 1995; Boskou, 2000; Food and Agricultural Organization, 2000). A daily consumption of 50 g olive oil with a concentration of 180 mg/kg of phenols would result in an estimated intake of about 9 mg of olive oil phenols per day. This is similar to the intake of flavonols and catechins from apples (about 10 mg/day), but lower than that of catechins from tea (50 mg/day) (Arts *et al*, 2001) or phenolic acids from coffee (200 mg/day) (Radtke *et al*, 1998). However, it is difficult to compare those intakes in terms of antioxidant activity because the antioxidant potential as well as the bioavailability might differ among various antioxidants. One way to get more insight into the potential of the antioxidants is to express the amount of dietary intake in moles rather than milligrams, because the antioxidant activity depends on the number of reactive OH groups. In six Greek olive oils, which were specifically analysed to measure the phenol content by HPLC, tyrosol and hydroxytyrosol comprised on average 10 weight% (range 5–16%) and aglycones 90 weight% (range 84–96%) (unpublished data, personal communication from Dr S van Boom). Based on these figures and assuming a phenol intake of 9 mg/day in Mediterranean countries, we can estimate that about 1 mg (6 μmol) is derived from hydroxytyrosol and tyrosol and about 8 mg (23 μmol) from the aglycones. Then, total phenol intake in the Mediterranean area would be about 29 μmol. However, this does not yet represent the total amount of effective olive oil phenols. Part of the phenols in olive oil are ligstroside-aglycones and tyrosol, which are *mono*-phenols (Figure 1) with little or no antioxidant capacity (Rice-Evans *et al*, 1996; Leenen *et al*, 2002). Of the six analysed Greek olive oils, the mean percentage of diphenols was 44 mol% (range 39–51 mol%). Thus, the intake of phenols with antioxidant capacity is about $0.44 \times 29 = 13$ μmol, which is equivalent to 2 mg of hydroxytyrosol per day. Thus, if the six Greek oils are considered as representative for the Mediterranean area, then the intake of antioxidants from olive oil in that area can be estimated to be 2 mg hydroxytyrosol-equivalents per day.

Methods

To identify studies on the health effects and metabolism of olive oil phenols, we searched the MEDLINE database (National Library of Medicine, Bethesda, MD, USA) for the years 1966–2002 using the following keywords: phenol*, polyphenol*, olive oil, tyrosol, hydroxytyrosol, oleuropein, antioxidant, oxidation, absorption, bioavailability, and metabolism. We also searched the ISI Web of Science Citation Databases for articles that cited two well-known publications on this topic (Visioli *et al*, 1995; Wiseman *et al*, 1996).

To address the bioavailability of olive oil phenols, we reviewed animal and human studies on the absorption, metabolism, and urinary excretion of olive oil phenols.

We selected human and animal intervention studies that examined the effect of consumption of olive oil phenols on oxidation markers in plasma. We excluded studies in which we could not disentangle the antioxidant effects of phenols from those of the fatty acid composition of olive oil, studies without a control diet, and studies in which the amount of ingested phenols was not reported or could not be estimated. Human studies were stratified according to measurement of oxidation markers in fasting *vs* postprandial blood.

We specifically extracted data on the lag time of LDL oxidisability and combined these in a random-effects model assuming heterogeneity (DerSimonian & Laird, 1986). To this end, we extracted or estimated for each study the differences in lag time between the high and low phenol treatment, and the s.e. of these differences. A model assuming equal sampling variances for each study, that is, each study having equal weight, and a fixed-effects model assuming homogeneity, yielded similar results.

Results

We found 13 publications that addressed absorption, metabolism, or urinary excretion of olive oil phenols: five animal (Bai *et al*, 1998; Coni *et al*, 2000; D'Angelo *et al*, 2001; Tuck *et al*, 2001; Visioli *et al*, 2001), six human studies (Bonanome *et al*, 2000; Visioli *et al*, 2000a, b; Miro-Casas *et al*, 2001a, b; Vissers *et al*, 2002), and two studies that addressed characterisation of the metabolites of hydroxytyrosol in urine from two of the above-mentioned studies (Caruso *et al*, 2001; Tuck *et al*, 2002). One study by our group (Vissers *et al*, 2002) showed that apparent *in vivo* absorption of the ingested olive oil phenols was more than 55–66 mol% in humans. A study in rats showed that bioavailability of radiolabelled tyrosol and hydroxytyrosol was 71–99% compared to intravenously administered tyrosol and hydroxytyrosol (Tuck *et al*, 2001). Eight studies showed that of the total amount of ingested phenols, at least 5% was excreted in urine as tyrosol and hydroxytyrosol (Visioli *et al*, 2000a, b, 2001; D'Angelo *et al*, 2001; Miro-Casas *et al*, 2001a, b; Tuck *et al*, 2001; Vissers *et al*, 2002), mainly as glucuronide (Visioli *et al*, 2000b; Miro-Casas *et al*, 2001a, b; Tuck *et al*, 2002) and monosulphate conjugates (D'Angelo *et al*, 2001; Tuck *et al*, 2002), and in the *O*-methylated form as homovanillic alcohol and homovanillic acid (Visioli *et al*, 2000a; Caruso *et al*, 2001; D'Angelo *et al*, 2001; Tuck *et al*, 2002).

A total of 11 published papers addressed the antioxidant effects of consumption of phenol-rich olive oil compared to consumption of phenol-poor olive oil. Seven human studies (Table 1) and four animal studies (Table 2) investigated the effects of olive oil phenols on oxidation markers in blood and urine. Five human studies compared the effects between phenol-rich and phenol-poor olive oil in fasting blood samples (Table 1a) and three in postprandial blood samples (Table 1b). We were unable to evaluate the effect of olive oil phenols on LDL oxidation in several recent animal and human studies (Masella *et al*, 2001; Ochoa-Herrera *et al*,

Table 1 Human studies on the antioxidant effect of olive oil phenols in fasting blood samples (a) and (b) in postprandial blood samples

Treatment	*Subjects (n)*	*Dose of phenols*	*Design*	*Oxidation marker*	*Result (high* vs *low phenol treatment)*	*Direction of phenol effect*[a]	*Ref.*
(a)							
Extra virgin olive oil *vs* oleic acid-rich sunflower oil	10	16 *vs* 0 mg/day[b]	3 weeks crossover	Lag phase (min)	59 *vs* 64	0/−	Nicolaiew *et al* (1998)
				Max. rate (μmol/min/g LDL protein)	11 *vs* 11	0	
				Total conjugated diene formation (μmol/g LDL protein)	483 *vs* 485	0	
Extra virgin *vs* refined olive oil	24	33 *vs* 3 mg/day	3 months crossover	Rate of TBARS formation ((nmol TBARS/mg LDL protein)/(μmol Cu^{2+}/l))	12 *vs* 15	+	Ramirez-Tortosa *et al* (1999)
		vit. E: 12 *vs* 8 mg/day		Macrophage uptake of oxidised LDL (% of LDL uptake by U937 macrophages)	35 *vs* 46	+	
Extra virgin *vs* refined olive oil	14	Tyrosol + hydroxy-tyrosol: 0.4 mg/day	1 month crossover	Lag phase (min)	40 *vs* 47	0/−	Bonanome *et al* (2000)
				Peroxidation rate (nmol O_2 uptake/min)	13 *vs* 15	0/+	
Phenol-rich *vs* phenol-poor extra virgin olive oil	46	21 *vs* 3 mg/day	3 weeks crossover	Lag phase:			Vissers *et al* (2001b)
				-In LDL (min)	109 *vs* 110	0	
				-In HDL (min)	70 *vs* 69	0	
				Max. rate:			
				-In LDL (μmol/min/g LDL protein)	12 *vs* 12	0	
				-In HDL (μmol/min/g HDL protein)	4.4 *vs* 4.6	0	
				Malondialdehyde (μmol/l)	0.7 *vs* 0.7	0	
				Lipid hydroperoxides (μmol/l)	0.4 *vs* 0.4	0	
				Protein carbonyls (nmol/mg protein)	0.2 *vs* 0.2	0	
				Ferric reducing ability of plasma (mmol/l)	1.1 *vs* 1.1	0	
Phenol-rich *vs* phenol-poor extra virgin olive oil	25	22 *vs* 3 mg/day	3 weeks crossover	Oxidation of plasma:			Moschandreas *et al* (2002)
				-Lag phase (min)	113 *vs* 111	0	
				-Max. rate (μmol/min)	0.2 *vs* 0.2	0	
				Malondialdehyde (μmol/l)	0.6 *vs* 0.6	0	
				Lipid hydroperoxides (μmol/l)	0.5 *vs* 0.7	0	
				Protein carbonyls (nmol/mg protein)	0.2 *vs* 0.2	0	
				Ferric reducing ability of plasma (mmol/l)	1.1 *vs* 1.1	0	
(b)							
Extra virgin olive oil *vs* oleic acid-rich sunflower oil	10	16 mg/day + 12 mg[b] on last day	3 weeks crossover, blood sampling 6 h after intake on last day	Δ compared to $t=0$[c]:			Nicolaiew *et al* (1998)
				Lag phase (min)	4 *vs* 3	0/+	
				Max. rate (μmol/min/g LDL protein)	−1 *vs* −1	0	
				Total conjugated diene formation (μmol/g LDL protein)	−30 *vs* −24	0/+	

Table 1 Continued

Treatment	*Subjects (n)*	*Dose of phenols*	*Design*	*Oxidation marker*	*Result (high vs low phenol treatment)*	*Direction of phenol effect*[a]	*Ref.*
Olive oil with different amounts of phenolic extract	6	24 mg 49 mg 73 mg 98 mg	Four single doses, 24 h urinary collection	Urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$	(pg/mg creatinine) 24 mg 49 mg 73 mg 98 mg	 +	Visioli *et al* (2000a)
Olive oil with extracts high in polar or nonpolar phenols *vs* olive oil low in phenols	12	100 *vs* 0 mg	Three single doses, blood sampling at 0, 30, 120 min	Δ compared to $t=0$[d]: Lag phase (min) $t=30$ min $t=120$ min Max. Rate (μmol/min/g LDL protein) $t=30$ min $t=120$ min	 4 *vs* 6 4 *vs* 8 0 *vs* –1 0 *vs* –1	 0/− 0/− 0 0	Vissers *et al* (2001a)

[a]0 = no effect, + = protective effect, − = negative effect, 0/+ = no significant effect in the protective direction, 0/− = no significant effect in the negative direction.
[b]Intake of olive oil and phenols was estimated from the amount of MUFA in the diet and in the oil. We assumed that all MUFA in the diet was derived from olive oil. This estimate is therefore the maximum olive oil intake per day.
[c]In the postprandial study of Nicolaïew *et al*, we subtracted the lag times of $t=0$ from the lag times at $t=6$, and then calculated the difference in lag time between consumption of the high and low phenol oil.
[d]In this study, the effects of three various supplements were compared with each other: one containing mainly nonpolar olive oil phenols; one containing mainly polar olive oil phenols; and one without phenols (placebo). The effects between the supplement that contained mainly nonpolar or polar olive oil phenols did not significantly differ. We, therefore, here present the mean lag-time and maximum rate of LDL oxidation after consumption of the supplements high in non-polar and polar phenols compared to $t=0$.

2001; Ruiz-Gutierrez *et al*, 2001; Fito *et al*, 2002; Gimeno *et al*, 2002; Ochoa *et al*, 2002), because we did not know the exact amount of ingested olive oil phenols, there was no control diet parallel to the phenol-rich diet, or antioxidant systems were studied in the liver instead of in plasma.

The four animal studies showed a lower LDL oxidisability, a higher concentration of vitamin E in plasma, or a lower concentration of oxidation products in urine after consumption of phenol-rich olive oil than after consumption of phenol-poor olive oil (Table 2). In five out of seven human studies, however, these effects of phenols were not found (Table 1a and 1b). The only oxidation marker that could quantitatively be combined across studies was the lag time of LDL oxidation (Figure 2). Although animal studies suggest a protective effect, human studies suggest that olive oil phenols reduced the lag time somewhat and thus increased rather than decreased LDL oxidisability. The combined estimate of the difference in lag time of LDL oxidisability between high and low phenol treatment was -3.7 ± 2.2 min (mean $\pm$ s.e.) (Figure 2).

Discussion

Bioavailability

The first requirement for a dietary compound to be a potential *in vivo* antioxidant in humans is that it enters the blood circulation. Animal and human studies show that olive oil phenols are well absorbed. Absorption is confirmed by the recovery of tyrosol and hydroxytyrosol in urine after intake of olive oil phenols. A further requirement for protection against oxidative LDL modification is that the ingested compound becomes available in plasma or LDL in a form with antioxidant capacity, because the original antioxidant activity of an absorbed compound may alter upon metabolism. Thus, insight into the kinetics and metabolism of olive oil phenols is needed to assess their potential for increasing the antioxidant capacity of LDL or plasma in the human body.

The amount and form in which the olive oil phenols are present in plasma or are excreted in urine may give insight into their metabolism in the human body. At present, data on plasma phenol concentrations that can be reached after consumption of olive oil are scarce. A recent study of Gimeno *et al* (2002) showed that total phenol concentration in LDL increased from 4.8 ± 1.8 ng/mg protein to 8.0 ± 2.3 ng/mg protein after subjects consumed 25 ml/day of olive oil or about 6 mg/day of phenols for 1 week. However, it was not clear if those phenols were specific for olive oil. Masella *et al* (2001) reported that the plasma concentration of total phenolic compounds significantly increased from 11.3 mg/l, expressed as tyrosol concentration, after 6-week consumption of 20 g/day of olive oil with a low content of phenols (11 mg/kg) to 23.0 mg/l after 4-week consumption of 20 g/day of extra virgin olive oil with a high content of phenols (238 mg/kg). However, those plasma concentrations are improbably high. If the plasma compartment is set at 3 l, then a daily extra

Table 2 Animal studies on the antioxidant effect of olive oil phenols

Treatment	*Species (n)*	*Dose of phenols*	*Duration*	*Oxidation marker*	*Result (high vs low phenol oil)*	*Direction of phenol effect*[a]	*Ref.*
Extra virgin olive oil *vs* refined olive oil and Trisun high oleic sunflower oil	Rabbits (24)	8.7 *vs* 0.1 *vs* 0 mg[c]	6 weeks consumption	Lag phase (min) Max. rate (μmol/min/g LDL protein) Malondialdehyde (nmol/l)	283 *vs* 218[b] 6.1 *vs* 6.4 610 *vs* 560[d]	+ 0/+ −	Wiseman *et al* (1996)
Olive mill waste water extract dissolved in ethanol *vs* ethanol	Rats (12)	Hydroxytyrosol 83 μg *vs* 0 μg	6 day consumption +4 days 20 min/day exposure to smoke, 24-h urine sample on day 0, 2, 4	Δ urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ compared to $t=0$ (pg/mg creatinine) Day 2 Day 4	 −4 *vs* 82 84 *vs* 145	 + 0/+	Visioli *et al* (2000c)
Olive mill waste water extract dissolved in water/ethanol solution *vs* water/ethanol solution	Rats (3)	Hydroxytyrosol 83 μg *vs* 0 μg	One single dose, blood sampling at 0, 15, 30, 90, 240 min	Plasma antioxidant capacity: $Cu^{2+} \rightarrow Cu^{+}$ with uric acid as reference (mEq uric acid): $t=0$ $t=15$ $t=30$ $t=90$ $t=240$	 205[e] 233 *vs* 210 198 *vs* 208 190 *vs* 210 250 *vs* 208	 0/+ 0/− 0/− +	Visioli *et al* (2001)
Extra virgin olive oil versus extra virgin olive oil from which phenols were removed	Hamsters (20)	151 *vs* 20 μg/day	5 weeks consumption	Lag phase (min) Max. rate (μmol/min/g LDL protein) Max. conjugated diene formation (nmol/l) Ferric reducing ability of plasma (mmol/l) Plasma vitamin E (μg/ml)	62 *vs* 47 4.9 *vs* 5.4 593 *vs* 544 0.51 *vs* 0.50 12.7 *vs* 10.1	+ 0 0 0 +	Wiseman *et al* (2002)

[a]0 = no effect, + = protective effect, − = negative effect, 0/+ = no significant effect in the protective direction, 0/− = no significant effect in the negative direction.
[b]Data on the refined olive oil and Trisun high oleic sunflower oil were combined because of their low phenol content.
[c]Estimated phenol intake (personal communication: R. Leenen and A. Roodenburg, Unilever Research Vlaardingen, the Netherlands).
[d]Plasma malondialdehyde was significantly reduced in the refined olive oil group.
[e]The values were estimated from the figures presented in the article of Visioli *et al* (2001).

intake of 4.76 mg/day would cause an increase of phenols in the plasma compartment of maximally 1.6 mg/l if phenols were absorbed immediately and dissolved completely, which is not the case in reality. For comparison, intake of 68 mg of quercetine equivalents produced a peak concentration of 0.2 mg/l (Hollman *et al*, 1997). Therefore, it is improbable that the concentration of olive oil phenols in plasma increased by more than 10 mg/l. Another study measured the amounts of hydroxytyrosol and tyrosol in LDL of humans, but the variability was too high to provide reliable estimates of concentrations in LDL (Bonanome *et al*, 2000). Bai *et al* (1998) found only low concentrations of plasma hydroxytyrosol concentrations in rats after oral administration of a high single dose of hydroxytyrosol. However, Bai *et al* analysed hydroxytyrosol without prior deconjugation, which may cause underestimation of total hydroxytyrosol in plasma. Likewise, Coni *et al* (2000) also analysed olive oil phenols without deconjugation in plasma of rabbits that consumed extra virgin olive oil for 6 weeks. Thus, reliable data on plasma concentration of olive oil phenols are scarce. An alternative is to look at olive oil phenols excreted in urine; these may provide information on the form in which phenols are present in plasma. The reported figure for the recovery of ingested olive oil phenols as tyrosol and hydroxytyrosol in urine in humans range between 5 and 72%, most of them conjugated to glucuronic acid (Visioli *et al*, 2000a, b; Miro-Casas *et al*, 2001a, b; Vissers *et al*, 2002). This wide range is probably due to the various approaches to calculate urinary excretion and to different analytical analyses. For instance, Visioli *et al* (2000b) measured the percentage recovery in urine of total ingested tyrosol and hydroxytyrosol without taking into account the production of additional hydroxytyrosol and tyrosol from ingested oleuropein- and ligstroside-aglycones in the body. Their reported recovery of 20–60% may thus be an

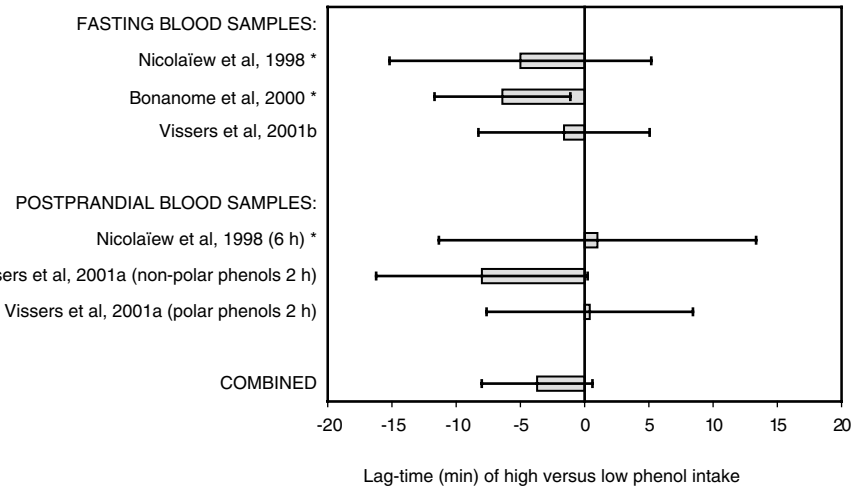

Figure 2 Effect of olive oil phenols on the lag time of LDL oxidisability compared to a control treatment of monounsaturated vegetable oil in humans. Bars are mean differences, and the lines are 95% CIs. We estimated the s.e. of the difference in studies with an asterisk by adding the variance of the groups consuming high or low phenol oil. In the postprandial study of Nicolaïew *et al*, we subtracted the lag times of $t=0$ from the lag times at $t=6$, and then calculated the difference in lag time between consumption of the olive oil and sunflower oil. CIs were calculated by 1.96 * s.e.

overestimate. Miro-Casas *et al* found a recovery for hydroxytyrosol of 72% of total ingested hydroxytyrosol-like substances, after acidic hydrolysis instead of enzymatic hydrolysis in the chemical analysis. They could therefore not provide specific information about the type of conjugate, and it is possible that other types of conjugated metabolites were present in urine (Miro-Casas *et al*, 2001b). We found a recovery of 5–16 mol% of total ingested phenols (Vissers *et al*, 2002), which is lower than that reported by others. However, our finding is probably an underestimate because we did not measure metabolites of olive oil phenols, such as *O*-methylated hydroxytyrosol (3-hydroxy-4-methoxyphenylethanol) in urine (Manna *et al*, 2000; Visioli *et al*, 2000a; Caruso *et al*, 2001; D'Angelo *et al*, 2001; Tuck *et al*, 2002). Taken together, data on urinary excretion indicate that at the very least 5% of ingested olive oil phenols is recovered in urine as (glucuronidated) tyrosol and hydroxytyrosol. The remaining phenols are probably metabolised into other compounds, such as *O*-methylated hydroxytyrosol. Monosulphate conjugates might be other metabolites as shown in two rat studies (D'Angelo *et al*, 2001; Tuck *et al*, 2002). Whether olive oil phenols are also metabolised into these conjugates in humans remains to be elucidated. Thus, olive oil phenols may be present in plasma mainly in the glucuronidated and *O*-methylated form. This suggestion needs to be confirmed by analyses of phenols and their metabolites in plasma. Therefore, development of methods to analyse these phenols in plasma is needed.

Studies on LDL oxidisability and other markers of oxidation

Animal studies suggest that olive oil phenols protect LDL against oxidation as indicated by decreased LDL oxidisability or other markers of oxidation (Table 2). In contrast, five human studies do not point to protective effects of olive oil phenols on LDL oxidisability. Our meta-analysis (Figure 2) suggested that the lag time of *ex vivo* LDL oxidation after high phenol treatment was 3.7 min lower (95% CI, −8.0 to 0.6 min) than after low phenol treatment, which does not suggest decreased susceptibility of LDL to oxidation.

Some other studies suggest a protective effect of olive oil phenols as indicated by markers of oxidation other than the lag time of LDL oxidation. Visioli *et al* (2000a) found that administration of phenol-rich oils resulted in a dose-dependent decrease in urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$, an F_2-isoprostane, which indicates less overall oxidation of arachidonic acid. Ramirez-Tortosa *et al* (1999) showed a decreased LDL oxidation rate as measured by thiobarbituric acid-reactive substances and a reduced *in vitro* uptake of oxidised LDL by macrophages in fasting blood after 3 months of consumption of extra virgin olive oil compared to refined olive oil. Furthermore, Bonanome *et al* (2000) found an increase of antioxidant capacity of postprandial plasma samples measured by a crocin-bleaching test 2 h after intake of 100 ml extra virgin olive oil. However, this study did not include a control group and the effect might therefore be due to a nonspecific meal or time effect. Thus, results of human studies on the effects of olive oil phenols on various markers of oxidation are inconsistent, with most studies showing no effect.

Are phenols antioxidants *in vivo*?

How can we explain that animal and *in vitro* studies suggest antioxidant effects of olive oil phenols, while most human studies do not find effects? Possible explanations include the dose and plasma concentration of phenols, type of oxidation marker, and metabolism of the phenols in the body. In

addition, we should consider the possibility of publication bias. We speculate that negative outcomes of human studies may have a higher chance of being published than negative outcomes of animal studies, either because human studies are more expensive and difficult, which increases the pressure to publish for investigators, or because they are more likely to be accepted for publication. We found only a few animal studies that specifically studied the antioxidant effect of consumption of phenol-rich olive oil compared to consumption of phenol-poor olive oil. It is conceivable that more animal studies have been carried out but remained unpublished because of negative results. On the other hand, it is quite possible that laboratory animals simply react differently to olive oil phenols than humans.

Differences in the doses of phenols fed might also explain discrepant results between animal and human studies. An important question is whether the doses and thus the plasma concentration of antioxidant phenols in humans was high enough to affect the markers of LDL oxidation. Unfortunately, reliable data on plasma concentration of olive oil phenols are lacking. We can estimate the maximum attainable plasma concentrations as follows. In all, 50 g/day of olive oil provides 2 mg or 13 μmol of hydroxytyrosol-equivalents per day. If these are absorbed immediately and completely dissolved into a plasma compartment of 3 l, this would result in a plasma concentration of 4.3 μmol/l. In reality, however, absorption is slow and incomplete, and clearance and redistribution will remove phenols from plasma immediately after absorption. We can make a more realistic guess at attainable plasma concentrations using data on other phenolic compounds. For instance, intake of 225 μmol of quercetin-glucosides (68 mg quercetin equivalents) from onions, which are absorbed for about 50% (Hollman *et al*, 1995), produced a peak concentration of 0.75 μmol of quercetin per litre plasma in humans (Hollman *et al*, 1997). This peak rapidly fell off in the first hours after consumption. By analogy, intake of 13 μmol of hydroxytyrosol-equivalents (2 mg), which is absorbed for 66% (Vissers *et al*, 2002), might produce a peak concentration of 0.06 μmol of hydroxytyrosol-equivalents per litre of plasma. In contrast, when olive oil phenols were added directly to plasma *in vitro*, concentrations of 50–100 μmol/l were required to protect LDL from oxidation (Leenen *et al*, 2002). This is several orders of magnitude higher than our estimate of the maximum concentration attainable after an intake of a high dose of olive oil phenols *in vivo*. Other *in vitro* studies demonstrated that preincubation of a purified LDL fraction with 10 μmol/l of olive oil phenols prevented oxidation (Grignaffini *et al*, 1994; Visioli *et al*, 1995; Caruso *et al*, 1999). However, phenols react avidly with many proteins, and therefore in whole plasma olive oil phenols will bind to other proteins like albumin, whereas in a purified LDL fraction these phenols can only bind to LDL. Hence, higher concentrations of olive oil phenols would be needed to protect LDL from oxidation in plasma than in a purified LDL fraction. Thus, it is likely that the plasma concentrations of olive oil phenols in the human studies were too low to affect LDL oxidisability. Our estimate of the *in vivo* plasma concentration is of course crude and subject to a number of errors, but it is unlikely to be several orders of magnitude too low, which is the difference between our estimate and the concentrations needed *in vitro*.

The actual concentration of phenols within the core of the LDL particle might be higher if phenols are lipophilic and accumulate inside lipoproteins. The mean water/lipid partition coefficient of the lipophilic phenols, that is, the aglycones, is about 0.7 (unpublished data, personal communications Dr S Van Boom), which implies that their concentration in the lipid core of LDL will be $1{:}0.7 = 1.4$ times higher than in the surrounding aqueous medium. However, the same partitioning occurs *in vitro*. Even if some phenols show a higher affinity for the inside of the LDL particles, the concentration in the aqueous medium evidently still needs to be about 50–100 μmol/l to produce inhibition of conjugated diene formation.

The use of different markers of oxidation might also explain discrepancies in results. Olive oil phenols might act as plasma antioxidant in ways other than dissolved in LDL particles. Visioli *et al* (2000a) measured the urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ instead of the LDL oxidisability. They found a negative correlation between intake of olive oil phenols and excretion of these markers of oxidative stress, which indicates less overall oxidation of arachidonic acid. The *ex vivo* LDL oxidisability is measured in LDL particles isolated from plasma by centrifugation. For this *ex vivo* analysis, it is necessary that all phenols in or attached to LDL are isolated and not lost during centrifugation. Tyrosol and hydroxytyrosol are polar and will not easily dissolve into LDL particles, but *in vivo* they might loosely bind to the surface of the LDL particle (Vinson *et al*, 1995). If so, tyrosol and hydroxytyrosol might get lost during the centrifugation of LDL (Carbonneau *et al*, 1997; Halliwell, 2000), and the *ex vivo* method would underestimate *in vivo* effects. Urinary excretion of F_2-isoprostanes might be a better marker because those F_2-isoprostanes are formed *in vivo*. However, F2-isoprostanes have, like other oxidation markers, not yet been validated as true predictor of coronary heart disease end points. It remains possible that phenols from olive oil decrease LDL oxidisability, but that the currently available markers are not suitable to measure such an effect. Besides, although oxidised LDL has been demonstrated in the atherosclerotic lesions of animals and humans, the role of such oxidation in cardiovascular disease is still unclear (Steinberg & Witztum, 2002). Thus, for definitive answers as to the health effects of olive oil phenols through their antioxidant capacity, we need to know how LDL is oxidised *in vivo* and we need markers of oxidative processes in the body that reliably predict disease risk.

The antioxidant capacity of conjugated or *O*-methylated metabolites of hydroxytyrosol might differ from that of the hydroxytyrosol itself. The radical scavenging potency of *O*-methylated hydroxytyrosol was similar and that of the

3-*O*-glucuronide conjugate was more potent than hydroxytyrosol *in vitro*, whereas the monosulphate conjugate of hydroxytyrosol was almost devoid of its radical scavenging activity (Tuck *et al*, 2002). *In vivo*, however, conjugates might also have less antioxidant activity than the ingested compounds, as was shown for quercetine-glucuronide compared to quercetin (Manach *et al*, 1998; Day *et al*, 2000), and *O*-methylated quercetin also showed less antioxidant activity as compared to quercetin (Manach *et al*, 1998; Yamamoto *et al*, 1999). To determine the true antioxidant activity of olive oil phenols *in vivo*, future studies should focus on the antioxidant activity of the metabolites actually present in plasma rather than on the *in vitro* antioxidant activity of the phenols as present in the olive oil.

The daily intake, bioavailability, and the amount of hydroxyl groups per molecule, and thus the antioxidant potential (Rice-Evans *et al*, 1996; Leenen *et al*, 2002), differ among various types of phenols. Therefore, it is difficult to compare the antioxidant effect of olive oil phenols *in vivo* with that of phenols from other foods, like wine, coffee, tea, onions, or apples. Nevertheless, intake of phenolic compounds from tea is some 50 mg/day (Arts *et al*, 2001) or that from coffee 200 mg/day (Radtke *et al*, 1998). This is much higher than the intake of 2 mg/day of hydroxytyrosol equivalents from olive oil in the Mediterranean diet. Since it is still uncertain whether intake of tea or coffee protect LDL against oxidation *in vivo*, even in relatively high amounts, it is unlikely that the intake of such a small amount of olive oil phenols alone can protect LDL against oxidation.

It is possible that olive oil phenols alone are not capable to protect LDL against oxidation, but that they act together with other antioxidants from the abundance of plant-based foods in the Mediterranean diet. Also, olive oil phenols might well have beneficial effects outside the realm of LDL oxidation (Visioli *et al*, 2002), such as improvement of endothelial function (Herrera *et al*, 2001) and inhibition of platelet aggregation (Petroni *et al*, 1995). However, such effects are outside the scope of this review.

Conclusion

Although the olive oil phenols are well absorbed, the amount of olive oil phenols in the diet is probably too low to produce a quantifiable and biologically significant effect on LDL oxidisability.

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Note added in proof

Recently, Miro-Casas *et al* demonstrated that intake of 25 ml of virgin olive oil containing 1.2 mg or 8 μmol hydroxytyrosol-equivalents produced a peak concentration of about 25 μg or 0.16 μmol of hydroxytyrosol-equivalents per litre of plasma (Clin Chem 2003;**6**:945–52). We estimated that intake of 50 ml of extra virgin olive oil corresponds with an intake of 2 mg or 13 μmol hydroxytyrosol-equivalents, which may lead to a peak concentration of 0.06 μmol of hydroxytyrosol-equivalents per litre plasma. Thus, the data of Miro-Casas *et al* show that our estimates are in a realistic range.

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