Absorption, Metabolism and Excretion of Phenols Derived from Olive Products

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ABSTRACT

The ageing population of many societies has been accompanied by an increase in the incidence of chronic diseases. At the same time, people are more interested in healthy dietary patterns and the use of dietary supplements. It is in this context that olive oil and olive leaf have attracted attention. Both products contain a number of biophenols that have been associated with bioactivity and positive health outcomes. Data indicate that the phenols are absorbed and metabolised and that a minor fraction of the ingested dose is excreted in the urine. This is a necessary pre-requisite to biological activity. However, their metabolic fate remains controversial. The outcomes of in vivo human studies are examined and contrasted with in vitro and animal studies. Furthermore, whether the bioactivity translates into physiological outcomes has not been established conclusively and will depend on development of suitable biomarkers of functionality.

Keywords: antioxidant, biophenol, health, leaf

INTRODUCTION

Epidemiological studies demonstrate that those populations with a high consumption of plant-based foods, such as fruits, vegetables and grains, exhibit a lower incidence of chronic disease. Of particular note is the association between the traditional Mediterranean diet and the low incidence of heart and cardiovascular diseases and some cancers (Simeonpoulos 2001; Martinez-González et al. 2002; Serra-Majem et al. 2006). While it is not clear what particular aspect of the traditional Mediterranean diet is protective, these findings have been attributed to dietary fibre, vitamins and minerals, and apparent ideal macronutrient ratios (Fraser 1994; Jenkins et al. 1998; Serra-Majem et al. 2006). Moreover, the specific mechanism(s) behind the apparent favourable health outcomes of this eating pattern are yet to be determined. Meanwhile, much interest has been evoked, and research performed, on the benefits of olive oil consumption.

Olive oil has been touted for its ability to positively affect LDL-cholesterol levels (Gimeno et al. 2002) and hence limit atherosclerotic and coronary heart disease development. Recently olive phenols demonstrated a favorable effect on triglyceride metabolism in a rat model (Oi-Kano et al. 2003). Epidemiological evidence also suggests an inverse association for cancer, in particular breast cancer, and olive oil consumption (Lipworth et al. 1997; Menendez et al. 2008). The health benefits of olive oil have been attributed to a favourable fatty acid composition (Beardsell et al. 2002) or, alternatively, to an antioxidant effect by the phenolic fraction which comprises < 1% of the oil (Bravo 1998; Craig et al. 1999; Tripoli et al. 2005). Phenolic compounds are ubiquitous in the plant kingdom as they are products of plant secondary metabolism from both the shikimate and acetate pathways (Parr et al. 2000). The most characteristic phenols of olives are the secoiridoids (Damtoft et al. 1993). More recently, the interest in olive oil consumption has been extended to include table olives (Kountouri et al. 2002; Puel et al. 2007) and the use of olive leaves (Malik et al. 2008) as dietary supplements. A method has even been proposed to enrich oils such as olive with olive leaf biophenols (Salta et al. 2007; Japon-Lujan et al. 2008).

Several questions arise in relation to the phenolic fraction of olive products: Do olive biophenols exhibit in vitro and in vivo antioxidant activity? Do they exhibit other bioactivities? If yes, does the antioxidant/bioactivity translate to a physiological effect? If so, does the physiological effect enhance health? The potential biological activity of biophenols per se is dependent on their bioavailability; that is, their capacity to be taken up by the body and reach systemic circulation unchanged. This review examines various aspects of the bioavailability and bioactivity of phenols derived from both olive oil and olive leaf.

OLIVE PRODUCTS AS SOURCES OF BIOPHENOLS AND BIOACTIVITIES

The phenolic fraction of olive oil is extremely complex and dependent on fruit cultivar and processing practices but includes hydroxytyrosol, tyrosol, oleuropein derivatives, caf-
fectic, vanillic acid, syringic acid, protocatechuic acid, and p-hydroxyphenylacetic acid (Visioli et al. 1998; Obied et al. 2005). A number of these compounds are known to exert a strong antioxidant effect in vitro (Speroni et al. 1998; Benavente-Garcia et al. 2000; Paiva-Martins et al. 2001; Franconi et al. 2006). Table olives have been shown to also be a good source of phenolic compounds, with the hydroxytyrosol content higher than in olive oil (Romero et al. 2006b). Phenolic content of the olive leaf depends on a number of factors (Japon-Lujan et al. 2006). Oleuropein concentration increases in the olive leaf during fruit maturation (Ortega-Garcia et al. 2008) but decreases in the fruit (and probably extracted oil) (Malik et al. 2006). Copper sprays used to control olive fungal diseases caused a decrease in total phe- nolic content of the treated leaves (Ferreira et al. 2007).

Olive leaf extracts are marketed as being beneficial for a number of conditions and have even been used to combat fevers especially those associated with malaria (Benavente-Garcia et al. 2000). Commercially, olive leaf extracts are available in powdered capsule form, in liquid tonics and also combined with other herbs and vitamins.

The bioactivity and health benefits of olive oil-derived phenols have been studied extensively and numerous reviews have been published. Specifically, functional effects on human wellbeing (Suja et al. 2001; Tripoli et al. 2005; Covas et al. 2006b), the effect on the cardiovascular system (Covas 2007) and antioxidant plus other biological activities (Visioli et al. 2002) and (Visioli et al. 2002; Yang et al. 2007) bioavailability (Vissers et al. 2004) have been examined in recent reviews. Antioxidant activity (Frankel et al. 2008) has received much attention and in vitro studies establish unequivocally the antioxidant potential of olive biophenols (Papadopoulos et al. 1991; Visioli et al. 1998; Caruso et al. 1999; Fito et al. 2000; Owen et al. 2000a; Owen et al. 2000b; Cabrini et al. 2001; Bendini et al. 2007; Lavelli 2007; Rietjens et al. 2007; Romani et al. 2007). For example, both hydroxytyrosol and oleuropein potently and dose-dependently inhibited copper sulfate induced oxida- tion of LDL at physiologically significant concentrations (Visioli et al. 1994, 1995). The protective effects of hydroxy- tyrosol are demonstrated through assessment of various oxi- dative stress biomarkers in human plasma with hydroxytyrosol prevented copper-sulfate induced isoprostane accumulation, with a decline in forma- tion of TBARS (Salami et al. 1995). Hydroxytyrosol inhibited in vitro platelet aggregation, and the production of arachidonic acid metabolites in human blood (Petroni et al. 1995). Similarly, antioxidant activity has been demonstrated in both animal, ex vivo (Ruiz-Gutiérrez et al. 1995; Manna et al. 1997, 2000; Venkatachalam et al. 2002; Del Boon et al. 2003; Somova et al. 2003; Manna et al. 2004; Al- Azzawie et al. 2006; Andreoudou et al. 2006; Puel et al. 2006; Puel et al. 2008) and cell culture (Hamdi et al. 2005) studies of biophenols. Such results are encouraging.

In vivo studies generally involve olive oil, typically virgin or extra virgin in recognition of the higher levels of phenols in these grades, or the extracted biophenols. In some cases, olive oil is added to a diet of other foods to be identified and characterized. Serum levels of HDL-cholesterol increased linearly with phenol content, while total cholesterol: HDL-cholesterol ratio and triglycerides decreased for all oils. Oxidative biomarkers (conjugated dienes, hydroxy fatty acids and circulating oxidized LDL) decreased linearly with the phenolic content of the oils. Phenolic content of the oils was quoted as total phenols, hydroxytyrosol content higher than in olive oil (Romero et al. 2006b). Data for individual biophenols were not pre-

In the case of in vivo human studies (e.g. Vissers et al. 2001) results are more confusing and controversial and yet randomized, controlled, double-blind clinical trials (level I evidence) and large cohort studies (level II evidence) (Covas et al. 2006b) are required to clearly establish health benefits. In a notable study, Vissers et al. (2004) identified 11 published studies: four multi-center studies that addressed the antioxidant effects of consumption of phenol-rich versus phenol-poor olive oil. Data for the various studies, covering the period 1996 to 2002, were tabulated to compare treatment, phenol dosage, experimental design and oxidation biomarkers. The trials showed diversity in terms of methodology, sample population (e.g. age, health status), control of diet, specificity of the biomarkers of oxidative stress, and measurement or not of biomarkers of the com- pliance of the intervention (Spencer et al. 2008). Some general observations are possible. The animal studies sug- gested that olive oil phenols protected LDL against oxida- tion (Vissers et al. 2004) whereas the human studies did not indicate protective effects of olive oil phenols on oxidisabi- lity. Indeed, there was a single oxidation biomarker, namely, lag time of LDL oxidation, that could be compared across studies and that observed traditional studies with an oxidant diet, hyperlipidemic, coronary heart disease patients more likely to show positive outcome), nature of the intervention (time, type, etc), correct choice of biomarker and end point (appropriate to stage of pathophysiology or hypothesis being tested). Covas et al. (2006b) made several recommendations in this regard for future studies. The tabulation was updated (Covas 2007) by the addition of four studies in 2006-2007 with a new tabulation of four stu- dies investigating anti-inflammatory effects of olive oil bi- phenols. However, the main conclusions from the original comparison have not changed.

Various explanations have been offered for the discrepancy between in vitro/animal studies and human trials. For example, the similarity of metabolism between animals and humans has been questioned (Visioli et al. 2003), and hence comparison between human and animal studies must be considered. Additionally, the duration of the study may be a determining factor: animal and human experimen- tal studies generally last less than a month. It may be that habitual dietary intake, and not acute experimental con- sumption, of olive biophenols is required for health out- comes. Furthermore, the effects of long-term ingestion of olive biophenols is required for health outcomes.

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sented although tyrosol and hydroxytyrosol were stated as the “2 major phenolic compounds.” The referenced paper (Owen et al. 2000b) illustrates the distinction between total phenols as measured by summation and total concentration of individual phenols. There is clearly a significant phenolic pool that is not included in the usual data. Potentially this includes a range of phenolic material including recently identified oleuropein oligomers found in olive pulp and pomace (Cardoso et al. 2006). The significant role of lig-nans such as pinocembrin in the total phenolic pool is also notable.

Studies have emphasised hydroxytyrosol and this can be attributed to three factors. It is the major component of olive oil and the emphasis is understandable on this basis alone. Moreover, it has a hydrophilicity/lipophilicity (Visi-oli et al. 2002) that gives it potential functionality in both aqueous and lipidal systems. However, the emphasis may also be a result of methodological considerations associated with its facile measurement as total hydroxytyrosol fol-low ing a hydrolysis step. In contrast to the significant body of literature on hydroxytyrosol, there are limited data exam-ining the contribution of olive leaf on health outcomes (Zarzuelo et al. 1991). However, interest in olive leaf (De Leonards et al. 2008) and notably oleuropein is increasing as seen in papers dealing with improved extraction tech-nologies (Japon-Lujan et al. 2006) and bioactivity (Andrea-dou et al. 2006; Giamarellos-Bourboulis et al. 2006; Puel et al. 2006). For instance, acute doxorubicin cardiotoxicity in rats expressed by the alteration of intracellular and periphery markers (e.g. catheine phosphokinase, creatine phosphokinase-MB, lactate dehydrogenase, aspartate amino-transferase and alanine aminotransferase) was successfully treated with oleuropein through suppression of oxidative and nitrosative stress (Andreadou et al. 2007).

An electron paramagnetic resonance and spectrophoto-metric study of oleuropein oxidation has been reported (Tzika et al. 2008). Kinetic autoxidation data were derived from the results. Moreover, oleuropein has been shown to bind to endogenous peptides and it has been calculated to adopt a closed conformation where its phenolic hydrogens bind to endogenous peptides and it has been calculated to adopt a closed conformation where its phenolic hydrogens

**End-point measures**

Antioxidant activity is just one of a vast range of potential bioactivities (Waterman et al. 2007) that includes anti-inflammatory, antiatherogenic, antibacterial (Bazoti et al. 2005; Medina et al. 2006; Fiti et al. 2008) and antifungal activities (Korukluoglu et al. 2006). Visioli et al. (2002) in reviewing the biological activities of olive oil biophenols distinguished in vitro studies of antioxidant activities and in vitro studies on enzyme modulation leading them to conclude that the biological activities of olive biophenols ex-tend beyond their antioxidant properties to include enzyme modulation and binding to cellular components. This con-clusion is now well accepted (Yang et al. 2007) and Visioli et al. (2002) and Obied et al. (2005) have tabulated the vari-ous aspects of the pharmacology of olive leaf.

The bioactivity of phenols may be exerted via interaction with food components (Kanner et al. 2001; Gorelik et al. 2008a; Ligumsky et al. 2008) in the gastrointestinal tract in which case antioxidant action and protein-binding capac-ity are probably important. A recent experiment tested the hypothesis that the stomach functioned as a bioreactor and the gastric fluid as a medium for further dietary component oxidation and antioxidation (Gorelik et al. 2008b). In rats with an intake of red meat and red wine, postprandial malondialdehyde levels declined in those consuming the mixture relative to those fed red meat alone. Moreover, a dual antioxidant/pro-oxidant behaviour of oleuropein has been demonstrated in vitro (Mazziotti et al. 2006). If this behaviour extends in vivo it can lead to formation of quinone derivatives which interact with DNA either forming covalent adducts or causing depurination. Such modifica-tions in critical genes can induce mutations. We need to ask the question; what has been demonstrated in vitro?

There are many definitions of bioactivity ranging from the very general (which would see every chemical as bio-active) (e.g. Miriam-Webster Dictionary) to much more restricted definitions in which a substance to be considered bioactive must impart a measurable biological effect at a physiologically realistic level that affects health in a bene-ficial way (Schrezenmeir et al. 2000). However, regardless of definition or the particular bioactivity, we cannot observe and measure a physiological impact. For example, oxidative stress is believed to be a component of disease development, in particular, atherosclerosis and cancer. In theory, characterisation of this stress comprising target macro-biomolecules, a stressor (usually one or more free radicals), and endogenous/exogenous antioxidants, could be achieved by measurement of any one or more of these compo-nents. However, measurement of antioxidant concentra-tions is useful but interpretation of the data is complicated as concentration does not equate with activity. On the other hand, methods for direct measurement of the reactive spe-cies and, particularly free radicals responsible for this stress, are of limited use in humans (for example, many potent reactive species only have a very short half-life). Moreover, only a small fraction of known reactive species induce pot-entially severe oxidative damage. Thus, the measurement of outcomes of oxidative damage is probably more meaningful. Biomarkers for this procedure would be useful and could serve as important tools in developing and assessing agents to decrease damaging oxidation, and hence disease deve-loment.

Established biomarker techniques are diverse and vary from measurement of blood pressure and vascular tone (Halliwell et al. 2004) to liver enzymes (Vissers et al. 2001). Techniques have also been developed to quantify oxidation products of macromolecules in body samples, the most common being cells, serum and urine but skin, sperm and tissue biopsies may also be used (Halliwell 1999). Measure-ments include malondialdehyde, lipid peroxides and protein carbonyls (Vissers et al. 2001). However, the most common of the more specific molecular biomarkers are those of lipid peroxidation and DNA oxidation, namely, F2α-isoprostane (8-iso-PGF2α) and 8-hydroxy-2-deoxyguanosine, respectively. The literature on these biomarkers is extensive and there are a number of excellent reviews (Halliwell 1999; Hermans et al. 2007; Hwang et al. 2007). There are no studies addressing the impact of olive leaf biophenol intake on such markers whilst a number of papers have been published on the impact of olive oil intake. For example, in-creasing concentrations of catecholic biophenols when compared to human human plasma was associated with decreased excretion of 8-iso-PGF2α (Visioli et al. 2000). Interestingly, the urinary levels of 8-iso-PGF2α inversely correlated with those of homovanillic alcohol (4-hydroxy-3-methoxyphenylethanol), a catechol-O-methyltransferase (COMT)-derived metabolite of hydroxytyrosol. The authors noted that the metabolised fraction of hydroxytyrosol may reflect the proportion of hydroxytyrosol entering into cellular compartments whereas the non-volatilized hydroxytyrosol excreted in urine may represent a less biologically relevant fraction. These data present the first direct experimental evidence of healthful effects of olive biophenols on humans. In a later study involving mildly dyslipidemic sub-jects, olive oil consumption (with high and low biophenol content) was not associated with increased urinary excretion of isoprostanes although there were favourable changes in levels of circulating plasma concentrations of markers of cardiovascular condition (Visioli et al. 2005). In another
in vivo study that involved healthy male subjects, dose-dependent urinary excretion of biophenols occurred after single bolus ingestion of olive oils containing variable levels of the biophenols (Weinbrenner et al. 2004). However, amounts of plasma oxidative markers did not change at postprandial state after administration of olive oil. In a study that compared the effect of regional diet on cancer incidence in Northern and Southern Europeans, olive oil consumption was negatively correlated with urinary levels of markers of DNA oxidation (Machowetz et al. 2007). However, the effect was not related to the biophenol content of the oil. Thus, the data are conflicting and recent work has suggested that current methods for measurement of biomarkers of oxidative status may be inappropriate (Rabovsky et al. 2006). Further development of suitable biomarkers and methods for their measurement coupled with availability of labelled biophenols of high purity will facilitate future investigations.

ASSABRATION, METABOLISM AND EXCRETION

In contrast with the number of studies devoted to examining the bioactivity and health benefits of olive products and biophenols there have been fewer studies of their absorption. The latter, critically, determines a compound’s bioavailability which is the first requirement for in vivo bioactivity.

The absorption, digestion, metabolism and elimination of biophenols may follow a number of pathways. The simplest pathway involves direct excretion of the unchanged biophenols in the faeces. Some biophenols may undergo hydrolysis in the stomach or intestine and be eliminated without further metabolism. In either case, absorption does not occur. Alternatively, absorption of the biophenols or a metabolite may occur across the small intestine, with uptake by the liver, entering systemic circulation. It is in the liver that any phase I metabolism will occur involving reduction, hydrolysis or, more commonly, an oxidation process. Phase II metabolism involving conjugation is also likely with the Phase I/II metabolites excreted in the urine via the kidneys. Additionally, the biophenols may be excreted via the kidney by way of enterohepatic circulation. This involves absorption of the biophenols across the large intestine due to action of microflora, and subsequent uptake by the liver. The various pathways are summarised in Fig. 1.

The phenolic acids and flavonoids such as quercetin glucosides and rhamnoglucosides (e.g. rutin) that are common to many fruits including olive have been studied extensively (Rechner et al. 2002; Scalbert et al. 2002; Manach et al. 2004; Scalbert et al. 2004; Ito et al. 2005; Manach et al. 2005; Williamson et al. 2005; Silberberg et al. 2005). The compounds are found in olive and olive leaves (Morton et al. 2000) and there is evidence for each of the above processes. The chemical structure of the phenolic acid or flavonoid determines the rate and extent of absorption (Scalbert et al. 2000). For instance, the position of glycosylation plays a significant role (Day et al. 1998). Regardless of the process by which they are initially absorbed, flavonoids undergo extensive metabolism prior to entry into systemic circulation. Metabolites in the faeces are found in olive and olive leaves (Morton et al. 2000) and there is evidence for each of the above processes. The chemical structure of the phenolic acid or flavonoid determines the rate and extent of absorption (Scalbert et al. 2000). For instance, the position of glycosylation plays a significant role (Day et al. 1998). Regardless of the process by which they are initially absorbed, flavonoids undergo extensive metabolism prior to entry into systemic circulation. Metabolites in the faeces are found in olive and olive leaves (Morton et al. 2000) and there is evidence for each of the above processes.

The process of initial absorption or transport of olive-specific biophenols has been reported but much work remains to be done. The molecular mechanism for transport of 14C-hydroxytyrosol, using differentiated model Caco-2 cell monolayers and the large bowel model, suggests that this is the case. Other studies showed that oleuropein degradation was pH dependent with degradation occurring at pH >7 but not at pH 5.2 (Edgecombe et al. 2000).

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Nutrition of olive biophenols. Kendall et al.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Absorption</th>
<th>Metabolism</th>
<th>Urinary excretion</th>
<th>Markers</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Hydroxytyrosol, 1-4 mg ingested; tyrosol, 1.8-7.0 mg ingested.</td>
<td>Postulated that tyrosol and hydroxytyrosol dose-dependently absorbed.</td>
<td>Higher doses of phenols increased their rate of conjugation with glucuronide.</td>
<td>Excreted in urine mainly as glucuronic; 20-28% and 30-60% ingested dose of tyrosol and hydroxytyrosol, respectively excreted. homovanillic alcohol excreted.</td>
<td>Urinary excretion</td>
<td>Visioli et al. 2000, 2002</td>
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<tr>
<td>Olive oil (tyrosol and hydroxytyrosol – measurement details not supplied).</td>
<td>Post-prandial absorption and incorporation into lipoproteins.</td>
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<td>Olive oil with different levels phenols.</td>
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<td>Olive oil.</td>
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<td>Oleuropein; polar supplement mainly hydroxytyrosol, tyrosol and oleuropein aglycone derivative; non-polar supplement mainly tyrosol and ligstroside aglycone derivative.</td>
<td>Estimated 55-66% ingested phenols absorbed in small intestine not colon (structure and polarity regulate absorption).</td>
<td>Data supported absorption of intact phenols. Oleuropein degraded in gut and absorbed as hydroxytyrosol.</td>
<td>Hydroxytyrosol present largely (ca. 65%) as glucuronic conjugate with less than 2% free compound. Phenolic compounds are the subject of an extremely extensive first-pass intestinal/hepatic metabolism.</td>
<td>Urinary amounts of hydroxytyrosol and 3-O-methyl-hydroxytyrosol increased in response to virgin olive oil ingestion.</td>
<td>Miró-Casas et al. 2003a</td>
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<td>Olive oil (hydroxytyrosol and 3-O-methylhydroxytyrosol measured; hydrolysis step.</td>
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<td>Olive oil (hydroxytyrosol and tyrosol only measured).</td>
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<tr>
<td>Olive oil (single dose or seven daily doses).</td>
<td>Absorption dependent on vehicle of administration.</td>
<td>High excretion of hydroxytyrosol suggested hydrolysis of oleuropein. 0.2-10 mg total phenols comprising 6.3% hydroxytyrosol, 5.3% tyrosol and 40% oleuropein aglycones.</td>
<td>Hydroxytyrosol and tyrosol excretion increased after single dose and short-term intake of olive oil. Levels of urinary tyrosol obtained after one week of sustained doses (25 ml/day) of virgin olive oil were lower than those obtained after a single 50 ml dose. Levels of urinary hydroxytyrosol same after both interventions. Method involved hydrolysis step, conjugates not measured.</td>
<td>Tyrosol excretion increased after oil consumption. Urinary levels and excretion profiles differed between men and women.</td>
<td>Covas et al. 2003</td>
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<td>Olive oil containing 2.4 mg oleuropein aglycone and 0.6 mg hydroxytyrosol.</td>
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<td>Olive oil with high, moderate and low phenolic content.</td>
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<td>Ex vivo (tyrosol, hydroxytyrosol, oleuropein).</td>
<td>Oleuropein not absorbed or metabolised in small intestine; likely to reach large intestine and be degraded by colonic microflora.</td>
<td>Extensive degradation of oleuropein by cultures of colonic microflora; products included hydroxytyrosol.</td>
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<td>Corona et al. 2006</td>
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<td>Olive oil with different levels phenols.</td>
<td>Not examined.</td>
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Looking specifically at oleuropein, an internal perfusion technique was developed to estimate its absorption in both iso-osmotic and hypotonic luminal conditions (Edgecombe et al. 2000). The influence of hepatic and renal metabolism that complicate quantitative evaluation of absorption...
hydroxytyrosol was present in plasma and urine in conjunction with methodological difficulties it appears that at least 98% of concentrations of hydroxytyrosol and 3-0-methylhydroxytyrosol as one of the main metabolites of hydroxytyrosol (Casas et al. 2001). Covas et al. (2000) showed that tyrosol binds LDL in vitro. Following a one month intervention involving consumption of olive oil (50 mL per day) there was no indication that tyrosol or hydroxytyrosol were absorbed efficiently enough to be measured in plasma lipoproteins (Bona-nome et al. 2000). However, based on an assumption of rapid absorption and turnover, postprandial measurements following administration of 100 g olive oil showed tyrosol and hydroxytyrosol in plasma LDL, HDL and chylomicrons, with concentrations peaking between 60 and 120 minutes. The authors proposed that the olive phenols were absorbed from the intestine, though not through a pathway dependent on chylomicron formation. Between-subject variability in biophenol absorption was high. Oleuropein and other conjugated forms were not measured but if hydrolysed following absorption, they could contribute to the tyrosol and hydroxytyrosol found in plasma. The profiles of the metabolites were not measured as the methodology incorporated an hydrolysis step.

In contrast, a number of metabolites of olive oil phenols were identified in LDL as hydroxytyrosol monoglucuronide, hydroxytyrosol monosulfate, tyrosol glucuronide, tyrosol sulfate and homovanillic acid sulfate (de la Torre-Carbot et al. 2006). Hydroxytyrosol monoglucuronide existed as two isomers differing in position of attachment of the glucuronide moiety (de la Torre-Carbot et al. 2007). The fact that these metabolites are able to bind LDL strengthens claims that these compounds act as in vivo antioxidants. The LDL-bound biophenols can exert antioxidant activity in the arterial intima where most LDL oxidation occurs in micro-domains sequestered from the richness of antioxidants present in plasma (Witzum 1994; Reaven et al. 1995). These papers (de la Torre-Carbot et al. 2006, 2007) contribute significantly to our knowledge of olive biophenol metabolism as the actual metabolites were characterised rather than hydrolysis products as measured and reported in many papers.

In vivo human data for the absorption and urinary excretion of hydroxytyrosol and tyrosol following ingestion of olive oil have been reported by a number of authors with similar results (Visioli et al. 2000; Visioli et al. 2001). Once again, urine samples with glucuronidase were subjected to enzymatic hydrolysis prior to measurement of metabolites. The authors postulated that the two biophenols were dose-dependently absorbed and excreted in urine as glucuronide conjugates. Dose-dependent absorption of these compounds has been reported elsewhere (Covas et al. 2003) and appears to now be accepted (Visioli et al. 2000; Saija et al. 2001; Covas et al. 2003). The amount of hydroxytyrosol and tyrosol excreted in urine relative to intakes was 30-60% and 20-22%, respectively. Conjugated forms of these phenols were rapidly degraded to three metabolites including hydroxytyrosol (Manna et al. 2000; Casas et al. 2001; Miró-Casas et al. 2003a) and appears to now be accepted (Visioli et al. 2000; Saija et al. 2001; Covas et al. 2003). The estimated hydroxytyrosol elimination half-life was 2.43 h based on the assumption of a monocompartmental model although the plasma concentration-versus-time curves showed that the pharmacokinetics may fit into a bicompart-

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tion on absorption and metabolism. In the case of tyrosol, urinary excretion peaked at 0-4 h after ingestion of virgin olive oil by male subjects with a 0-8 h peak for females (Covas et al. 2003). Urinary recoveries of tyrosol and hydroxytyrosol were 18-20% (Covas et al. 2003) and 79-122% (Miró-Casas et al. 2003b) of ingested dose, respectively with some variation between single dose and sustained dose intake. The authors concluded that there were differences in the metabolism of the two phenols although other dietary or metabolic factors may have accounted for the observed differences (Miró-Casas et al. 2003b). Vissers et al. (2004) reported the recovery of olive biophenols from five studies as ranging between 5 and 72%. The wide range was attributable to different analytical methods and to various approaches to calculating urinary excretion.

Vissers et al. (2002) found that ileostomy subjects (that is those with a completely removed colon) excreted minimal quantities of olive biophenol in ileostomy effluent. Subjects consumed single doses of three different supplements: nonpolar supplement comprising mainly a ligstroside-aglycone derivative with small quantity of tyrosol; polar supplement comprising hydroxytyrosol with lesser amounts of tyrosol and an oleuropein-aglycone derivative; and an oleuropein supplement. The authors surmised from the low excretion into ileostomy effluent that a large proportion of ingested biophenols was absorbed. It was calculated that 55-73 mol% of the ingested amount was absorbed and that 5-16 mol% was re-excreted as tyrosol and hydroxytyrosol in urine. The method incorporated an hydrolysis step and so did not distinguish between free and conjugated biophenols.

Absorption rates for the biophenols were similar in ileostomy subjects and those with an intact colon (Vissers et al. 2002). This suggests that olive biophenols are absorbed mainly in the small intestine rather than in the colon. The authors hypothesized that oleuropein and oleuropein- and ligstroside-aglycones might be split into hydroxytyrosol or tyrosol and elenolic acid either in the gastrointestinal tract before they are absorbed or in the intestinal cells, blood or liver after absorption. From ex vivo stability data it was concluded that the latter situation was most likely. However, analytical limitations limit the conclusions about the absorption of these compounds.

When excretion data are examined closely with due allowance for the contribution of more complex biophenols to the urinary excretion pool, it is apparent that the entire intake is not excreted in the urine. The quantity not absorbed and that accumulated in organs or erythrocytes remains to be established for both single dosage and prolonged intake. There are few data for intracellular uptake in humans but in bovine erythrocytes, oleuropein uptake occurred with transport across the membranes giving access to intracellular sites (Saija et al. 2001). This is critical for certain bioactivities.

Methodological problems limit the conclusions from many bioavailability studies. Many studies incorporated an hydrolysis step in the metabolite analysis to convert phenolic glycosides and conjugates to aglycones thereby simplifying chromatograms and enhancing sensitivity. However, this approach destroys information on metabolite profiles and limits our understanding of the metabolic processes. In comparing their results with previous data, Vissers et al. (2002) noted the impact of various methodological differences on analytical data. Tuck et al. (2001) concluded that differences between their data and previous data could be a result of different handling of the phenols in humans and rats or, alternatively, to method-imposed limitations in previous studies. Other data have established that the rat model

\[ \text{Fig. 2 Proposed pathway for the in vivo metabolism of hydroxytyrosol (analogous metabolites are derived from tyrosol, 4-hydroxyphenylethanol).} \]
is not reflective of human metabolism (Visioli et al. 2002). Interpretation of data is further complicated as hydroxytyrosol, the most widely studied olive phenol, is also a well-known metabolite of dopamine (Miró-Casas et al. 2003a). Despite these limitations, from the information that has been presented, we can postulate an enzymatic pathway for the in vivo metabolism of both hydroxytyrosol and tyrosol (Fig. 2) (Tuck et al. 2002) in agreement with those previously reported. In the case of oleuropein, it has been stated (Miró-Casas et al. 2003a) that “oleuropein has been shown to be metabolized in the body and recovered in urine, mainly in the form of hydroxytyrosol.” The original paper (Vissers et al. 2002) noted that oleuropein was the only component from olives that could be supplied in a food grade pure form. However, supplements are generally not pure and it is likely that this material contained other bio-phenols as the oleuropein content was less than 3% by mass of the 1.9 g supplement administered. Such difficulties complicate interpretation of data from this paper with respect to metabolism of oleuropein. However, we can present a tentative pathway for its metabolism in the human body (Fig. 3).

We have emphasised the role of the parent biophenols based on a tacit assumption that parent metabolites are the potentially bioactive entities. However, some Phase II metabolites are more pharmacologically active than the parent compound as in the case of morphine (Hu 2007). This has not been investigated in the case of olive biophenols.

CONCLUSION

There is convincing evidence for the absorption and intracellular uptake of at least some olive biophenols in humans. This suggests a potential role for olive oil and olive leaf biophenols and, in particular, hydroxytyrosol and oleuropein. Positive effects on cardiovascular, glycemic and osteopenic processes have been demonstrated in animal models and epidemiological evidence suggests a positive role of these biophenols in human health. Further research into the effects of the olive biophenols is necessary to confirm their role. This should involve multi-disciplinary intervention studies that incorporate detailed investigations of the fundamental chemistry and bioavailability of these compounds. As with other antioxidants, establishing a clear effect is limited by the current lack of standardised biomarkers.

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