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.
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.

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 (Martinez-Gonzalez et al. 2002; Serra-Majem et al. 2006; Simopoulos 2001). 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. Epidemiological evidence also suggests an inverse association for cancer, in particular breast cancer, and olive oil consumption (Lipworth et al. 1997). 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 0.4-5% 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). More recently, the interest in olive oil consumption has been extended to include 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 (Japon-Lujan et al. 2008; Salta et al. 2007).

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 with lesser amounts of tyrosol, oleuropein derivatives, caffeic acid, vanillic acid, syringic acid, protocatechuic acid, and p-hydroxyphenylacetic acid (Obied et al. 2005; Visioli et al. 1998). A number of these compounds are known to exert a strong antioxidant effect in vitro (Benavente-Garcia et al. 2000; Franconi et al. 2006; Paiva-Martins et al. 2001; Speroni et al. 1998). The leaves of the olive tree (Silva et al. 2006) and small branches (Japon-Lujan et al. 2007) while not commonly consumed, contain many of the same phenolic antioxidants that occur within the oil but in much higher concentrations. These include oleuropein, demethyloleuropein, ligstroside, oleurosides, oleuropein aglycone, tyrosol, hydroxytyrosol, syringic acid, gallic acid and ferulic acid (Briante et al. 2002; Di Donna et al. 2007). The phenolic content of the 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 phenolic content of the treated leaves (Ferreira Icfr et al. 2007). Olive leaf extracts are marketed as being beneficial for a number of conditions and have been used traditionally 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 (Covas et al. 2006b; Saija et al. 2001; Tripoli et al. 2005),
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 (Bendini et al. 2007; Cabrini et al. 2001; Caruso et al. 1999; Fito et al. 2000; Lavelli 2007; Owen et al. 2000a; Owen et al. 2000b; Papadopoulos et al. 1991; Rietjens et al. 2007; Romani et al. 2007; Visioli et al. 1998). For example, both hydroxytyrosol and oleuropein potently and dose-dependently inhibited copper sulfate induced oxidation of LDL at physiologically significant concentrations (Visioli et al. 1994; Visioli et al. 1995). The protective effects of hydroxytyrosol are demonstrated through assessment of various oxidation biomarkers. Pre-incubation of LDL from human plasma with hydroxytyrosol prevented copper-sulfate induced isoprostane accumulation, with a decline in formation 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 (Al-Azzawie et al. 2006; Andreadou et al. 2006; Coni et al. 2000; Del Boccio et al. 2003; Manna et al. 1997; Manna et al. 2004; Puel et al. 2006; Puel et al. 2004; Ruiz-Gutiérrez et al. 1995; Somova et al. 2003; Tuck et al. 2001) 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, the olive oil phenols are identified and quantified although this frequently involves a hydrolysis step thereby restricting the information content.
Thus, when hydrolysis is employed oleuropein is not measured and although a minor component of most olive oils, it can be a significant contributor to the phenol content in some cases (Miro-Casas et al. 2003b; Tripoli et al. 2005). In other instances, oils with varying levels of phenols (usually designated low, medium, and high or phenol-rich/phenol-poor) are examined. Studies of antioxidant activity typically look for changes in oxidative status of the test individuals following ingestion of the oil or extracted biophenols.

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 papers (seven human studies; four animal 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 compliance of the intervention (Spencer et al. 2008). Some general observations are possible. The animal studies suggested that olive oil phenols protected LDL against oxidation (Vissers et al. 2004) whereas the human studies did not indicate protective effects of olive oil phenols on oxidisability. Indeed, there was a single oxidation biomarker, namely, lag time of LDL oxidation, that could be compared across studies and this suggested that olive biophenols enhanced rather than decreased LDL oxidisability. A more recent comparison (Covas et al. 2006b) tabulated results of nine human trials for the period
1998 to 2005, with four of the studies common to the earlier tabulation (Vissers et al. 2004). Outcomes of the comparison were similar to those previously reported and it appears, in the case of human studies, that a positive outcome, seen as a change in oxidative status, depends on the population (male, elderly, low antioxidant 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. Covas et al. (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 studies investigating anti-inflammatory effects of olive oil biophenols. 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 cautioned. Additionally, animals can be fed over 2 g/kg body weight of olive biophenols without toxic side-effects (D'Angelo et al. 2001); this is much more than humans generally consume. The duration of the study protocol may also be a determining factor: animal and human experimental studies generally last less than a month. It may be that habitual dietary intake, and not acute experimental consumption, of olive biophenols is required for health outcomes to be affected. Therefore, both the concentration of consumed olive biophenols and the study durations must be considered. The most significant study to date in relation to the effects of olive oil phenols on cardiovascular health was reported by Covas et al. (2006a). This involved a randomized, crossover, controlled study of 200 healthy adult males from several
countries consuming olive oil over three weeks with low (2.7 mg/kg), medium (164 mg/kg) and high (366 mg/kg) biophenol content. 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 based on HPLC measurement of tyrosol and hydroxytyrosol as “simple forms or conjugates” (Covas et al. 2006b; Owen et al. 2000b). Data for individual biophenols were not presented 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. This includes a range of phenolic material including recently identified oligomers (Cardoso et al. 2006). The significant role of lignans such as pinoresinol 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 (Visioli et al. 2002) that gives it potential functionality in both aqueous and lipoidal systems. However, the emphasis may also be a result of methodological considerations associated with its facile measurement as total hydroxytyrosol following a hydrolysis step. In contrast to the significant body of literature on hydroxytyrosol, there are limited data examining the contribution of olive leaf on health outcomes (Zarzuelo et al. 1991). However, interest in olive leaf (De Leonardis et al. 2008) and notably oleuropein is increasing as seen in papers dealing with improved extraction methodologies (Japon-Lujan et al.)
2006) and bioactivity (Andreadou 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 peripheral markers (e.g. creatine phosphokinase, creatine phosphokinase-MB, lactate dehydrogenase, aspartate aminotransferase and alanine aminotransferase) was successfully treated with oleuropein through suppression of oxidative and nitrosative stress (Andreadou et al. 2007).

An electron paramagnetic resonance and spectrophotometric 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 form a hydrogen bond network with the hydroxyl groups of the glucose moiety (Gikas et al. 2007). An understanding of its conformation may ultimately shed light on its mechanism of action. Interestingly, oleuropein exhibited anti-HIV activity by blocking the HIV virus entry to host cells (Lee-Huang et al. 2007a; Lee-Huang et al. 2007c). However, it was hydroxytyrosol that was identified as the main moiety for binding to HIV-1 fusion protein gp41 (Bao et al. 2007; Lee-Huang et al. 2007b).

**End-point measures**

Antioxidant activity is just one of a vast range of potential bioactivities (Waterman et al. 2007) that includes antiinflammatory, antiatherogenic, antibacterial (Bazoti et al. 2005; Medina et al. 2006) 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 extend beyond their antioxidant properties to include enzyme modulation and binding to
cellular components. This conclusion is now well accepted (Yang et al. 2007) and Visioli et al. (2002) and Obied et al. (2005) have tabulated the various reported bioactivities of olive biophenols.

The bioactivity of phenols may be exerted via interaction with food components (Gorelik et al. 2008a; Kanner et al. 2001; Ligumsky et al. 2008) in the gastrointestinal tract in which case antioxidant action and protein-binding capacity 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), with a positive outcome. 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 modifications in critical genes can induce mutations. We need to ask the more fundamental question; what is bioactivity?

There are many definitions of bioactivity ranging from the very general (which would see every chemical as bioactive) (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 beneficial way (Schrezenmeir et al. 2000). However, regardless of definition or the particular bioactivity, we must be able to 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 components. In practice,
measurement of antioxidant concentrations 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 species 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 potentially 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 development.

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). Measurements 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-isoPGF2α) 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, increasing concentrations of catecholic biophenols when administered to healthy male human volunteers resulted in decreased excretion of 8-iso-PGF2α (Visioli et al. 2000). Interestingly, the urinary
levels of 8-iso-PGF2 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-metabolized 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 subjects, 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 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.
ABSORPTION, 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 (Ito *et al.* 2005; Manach *et al.* 2004; Manach *et al.* 2005; Rechner *et al.* 2002; Scalbert *et al.* 2004; Scalbert *et al.* 2002; Silberberg *et al.* 2006; Williamson *et al.* 2005). These compounds are found in olive oil 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. This commences in the oral cavity where hydrolysis of glycosides may occur although with significant inter-individual variation (Walle et al. 2005). Those biophenols that reach systemic circulation are subjected to action by the liver, including Phase I and II metabolism (Fig. 2) (Rechner et al. 2002). Biophenol structure affects the level of Phase II conjugation with methyl, sulfate and glucuronide groups. Those biophenols that are not absorbed over the small intestine are taken to the large bowel. Colonic microflora may degrade more complex biophenols to simpler compounds such as phenolic acids, which may then be absorbed and hence, become part of the cyclic enterohepatic circulation (Scalbert et al. 2000). This degradation of biophenols by the colonic microflora (Fig. 3) (Rechner et al. 2002) may be more important for bioavailability than initially believed. After both oral and iv-doses of 14C-labelled quercetin a substantial proportion of the dose was metabolized into 14C–carbon dioxide presumably by microflora in the large intestine (Walle et al. 2001). Very large inter-individual differences are observed in the plasma concentrations and amounts of the biophenol metabolites excreted in urine mirroring significant individual variation in absorption and metabolism (Rechner et al. 2002). This variability has an important consequence for studies of the health impact of dietary phenol intake since this level of variability requires a very large population to demonstrate efficacy (Hu 2007).

Fewer data exist for the absorption and metabolism of the more characteristic olive biophenols (Table 1) such as tyrosol, hydroxytyrosol and oleuropein (Fernandez-Bolanos et al. 2008) and there are little data for their stability in the
stomach or their biotransformation in the colon (Corona et al. 2006). It is well established that in vitro transformation of oleuropein into glucose and oleuropein aglycone is readily achieved by β-glycosidases (Ranalli et al. 2006) and various acidic and alkaline treatments (Miro-Casas et al. 2003a; Ryan et al. 2001). Esterolysis of the oleuropein aglycone produces hydroxytyrosol. The assumption that endogenous or exogenous enzymes can produce the same outcome in vivo (Ranalli et al. 2006) is common. Furthermore, acidic hydrolysis is incorporated in a number of procedures to mimic gastrointestinal conditions during ingestion of olive oil (Miro-Casas et al. 2003a). “High molecular weight” olive biophenols were hydrolysed under conditions that simulated the gastric environment (Corona et al. 2006) although the structure of these components was not investigated. In contrast, Vissers et al. (Vissers et al. 2002) demonstrated that hydroxytyrosol and oleuropein were stable ex vivo in gastric juices and duodenal fluid for up to two hours. Our work 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).

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 seemed to occur via a passive diffusion with an intestinal transport system that was not saturable (Manna et al. 2000). The apparent permeability coefficients (Papp) for apical↔basolateral transport of hydroxytyrosol were similar indicating that the intestinal transport of hydroxytyrosol was bidirectional. Tuck and Hayball (Tuck et al. 2002) concluded from the magnitude of the calculated Papp that absorption of hydroxytyrosol in humans should be 100%. The only labelled metabolite arising from hydroxytyrosol was homovanillic alcohol which is a product of intestinal COMT.
activity (Manna et al. 2000). For glycosylated biophenols, conflicting evidence exists for their absorption across the brush border of the small intestine. It could be that these compounds are degraded to aglycones by the β-glucosidase enzymes to allow for passive diffusion of these substances (Scalbert et al. 2000). Otherwise, evidence also exists for the ability of glucose transporters to absorb these compounds, sugar moiety intact, over the brush border (Hollman et al. 1995).

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 (Stretch et al. 1999) were excluded by this process. The permeability of oleuropein in an iso-osmotic intestinal lumen was similar to that of clinically used drugs such as atenolol and classifies oleuropein as a poorly permeable compound. Any absorption of oleuropein under these conditions occurs predominantly via transcellular passive diffusion (despite its polarity) or paracellularly (despite its large size). Permeability was significantly greater under hypotonic conditions and it was postulated that this increase was due to an increase in paracellular movement which was facilitated by opening of the paracellular junctions. It was concluded that oleuropein is capable of permeating the intestine but the amount of oleuropein that reaches the systemic circulation unchanged is likely to be small. However, the validity of this model for humans in vivo is unclear and orally ingested oleuropein in an oily matrix might be absorbed better.

Studies in the rat show that biophenols such as hydroxytyrosol are converted enzymatically into four oxidized and/or methylated derivatives (D'Angelo et al. 2001). However, it has been claimed that excretion, at least in the case of hydroxytyrosol, differs between humans and the rat (Visioli et al. 2003).
Alternatively, cell culture studies have provided useful information. Corona *et al.* (2006) used a Caco-2 cell model, perfused rat intestinal model, simulated gastric juices and colonic microflora fermentations to study the decomposition of olive biophenols in the stomach, their absorption and metabolism in the small intestine and their biotransformation by the microflora of the large intestine. Using the rat intestinal model, oleuropein was not absorbed across either small intestinal segments (jejunum or ileum). In contrast, hydroxytyrosol and tyrosol were rapidly absorbed in both jejunum and ileum and significant amounts of both Phase I and II metabolites were identified in the serosal fluid. These comprised hydroxytyrosol, homovanillic alcohol plus glucuronides of both compounds and tyrosol, tyrosol glucuronide plus another unidentified glucuronide. The apparent permeability coefficients for both parental compounds indicated that they are both well absorbed from the intestine. In agreement with the rat small intestinal studies, there was no significant AP(mucosal)→BL(serosal) transport of oleuropein in the Caco-2 cell model. Enterocyte-mediated absorption and metabolism of hydroxytyrosol and tyrosol also occurred in the Caco-2 model. In contrast to the small intestinal model, the majority (90%) of the hydroxytyrosol appeared on the basolateral side as unmetabolized hydroxytyrosol with no glucuronidation. The remaining 10% was present as either homovanillic alcohol or a glutathionyl conjugate of hydroxytyrosol. The latter may be formed via the action of glutathione S-transferase or via oxidative metabolism of hydroxytyrosol followed by its reaction with glutathione. Tyrosol absorption rate was 60% independent of concentration whereas hydroxytyrosol absorption varied from 35 to 58% with concentration. Once again, permeability data suggested that both tyrosol and hydroxytyrosol are well absorbed. As oleuropein was not absorbed in the small intestine it was concluded that it most likely reaches the large intestine. Indeed, when
applied to a culture of colonic microflora, oleuropein was rapidly degraded to three metabolites including hydroxytyrosol and two unknown compounds.

Insight into the metabolism of biophenols can be obtained from a knowledge of the amount and form in which they are found in plasma (Bai et al. 1998; Coni et al. 2000; Ruiz-Gutierrez et al. 2000; Visioli et al. 2000). Plasma concentrations of hydroxytyrosol and 3-O-methylhydroxytyrosol increased following intake of virgin olive oil in a single dose (Miro-Casas et al. 2003a) with both compounds present as conjugates. Although calculations were complicated by methodological difficulties it appears that at least 98% of hydroxytyrosol was present in plasma and urine in conjugated forms, mainly glucuronates, suggesting extensive first-pass intestinal/hepatic metabolism of ingested hydroxytyrosol. Hydroxytyrosol and 3-O-methylhydroxytyrosol appeared rapidly in plasma, reaching maximum concentrations at 30 and 50 min, respectively post-oil ingestion. 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 bicompartamental model. Previous estimations from urinary data suggested a half-life of 8 h (Miro-Casas et al. 2001). The data (Miro-Casas et al. 2003a) confirmed 3-O-methylhydroxytyrosol as one of the main metabolites of hydroxytyrosol (Caruso et al. 2001).

Covas et al (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 (Bonanome 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 would 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 \textit{in vivo} antioxidants. The LDL-bound biophenols can exert antioxidant activity in the arterial intima where most LDL oxidation occurs in microdomains sequestered from the richness of antioxidants present in plasma (Reaven et al. 1995; Witztum 1994). 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.

\textit{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 (Casas et al. 2001; Miro-Casas et al. 2001; Visioli et al. 2000). For example, the \textit{in vivo} affects of hydroxytyrosol were examined in humans (Visioli et al. 2000) following ingestion of phenol-poor olive oil and the same oil enriched
with hydroxytyrosol and tyrosol. Urinary levels of unconjugated tyrosol and hydroxytyrosol correlated with their intake except at the highest dose. However, correlations were complete following treatment of urine samples with glucuronidase. 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 (Covas et al. 2003; Saija et al. 2001; Visioli et al. 2000). The amount of hydroxytyrosol and tyrosol excreted in urine relative to intakes was 30-60% and 20-22%, respectively. However, these proportions were calculated from the glucuronidase-hydrolyzed urines. Based on reports of the finding of homovanillic alcohol in human Caco-2 cells incubated with hydroxytyrosol (Manna et al. 2000), the urine samples analysed by Visioli et al. (Visioli et al. 2000) were re-examined and homovanillic alcohol and homovanillic acid were present (Caruso et al. 2001). Once again, urine samples were subjected to enzymatic hydrolysis prior to measurement of metabolites.

Urinary excretion data are another source of information 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% (Miro-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 (Miro-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 attributed 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. Moreover, other components such as oleuropein were not measured in the urine.

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.

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 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 (Miro-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. 4) (Tuck et al. 2002) in agreement with those previously reported. In the case of oleuropein, it has been stated (Miro-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 biophenols 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. 5).

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 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.
<table>
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<tr>
<th>Compound</th>
<th>Absorption</th>
<th>Metabolism</th>
<th>Urinary excretion</th>
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<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 glucuronide; 20–28% and 30–60% ingested dose of tyrosol and hydroxytyrosol, respectively excreted. homovanillic alcohol excreted</td>
<td>Urinary excretion F2-isoprostanes inversely related to phenol ingestion</td>
<td>Visioli et al. 2000; Visioli et al. 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>LDL oxidizability and total plasma antioxidant capacity</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>For all treatments: 5-16% ingested phenols excreted as hydroxytyrosol or tyrosol. Oleuropein- and ligstroside aglycones or glycosides not measured. Method involved hydrolysis step, conjugates not measured.</td>
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<td>Visser &amp; al. 2001</td>
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<tr>
<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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</table>

Table 1 Studies of the absorption, metabolism and excretion of olive biophenols.
| Olive oil (single dose or seven daily doses) | Tyrosol excretion increased after oil consumption. Urinary levels and excretion profiles differed between men and women. | Covas et al. 2003 |
| Olive oil containing 2.4 mg oleuropein aglycone and 0.6 mg hydroxytyrosol | Absorption dependent on vehicle of administration | High excretion of hydroxytyrosol suggested hydrolysis of oleuropein | 44% ingested hydroxytyrosol+homovanillic alcohol excreted; 234% ingested free hydroxytyrosol excreted; hydrolysis step in method limits conclusions. | Visioli et al. 2003 |
| Olive oil with high, moderate and low phenolic content | 0.2 – 10 mg total phenols comprising 6.3% hydroxytyrosol, 5.3% tyrosol and 40% oleuropein aglycones | Dose dependent excretion of tyrosol, hydroxytyrosol and 3-O-methylhydroxytyrosol | No change in oxidative stress biomarker concentrations | Weinbrenner et al. 2004 |
| Ex vivo (tyrosol, hydroxytyrosol, oleuropein) | Oleuropein not absorbed or metabolised in small intestine; likely to reach large intestine and be degraded by colonic microflora | Extensive degradation of oleuropein by cultures of colonic microflora; products included hydroxytyrosol | | Corona et al. 2006 |
| Olive oil with different levels phenols | Not examined | Not examined | Not examined | Various markers | Covas et al. 2006a |
Fig. 1 Possible routes for ingested biophenols.

Figure 2. Proposed mechanism for the in vivo metabolism of flavonols and hydroxycinnamic acids.

Figure 3. Proposed mechanism for the formation of hippuric acids involving degradation of biophenols by colonic microflora.

Figure 4. Proposed pathway for the in vivo metabolism of hydroxytyrosol and tyrosol.

Figure 5. Proposed pathway for the in vivo metabolism of oleuropein. (de la Torre-Carbot et al. 2007)
References


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