Author: M. Kendall, M. Batterham, P. D. Prenzler, D. Ryan and K. Robards

Title: Absorption, metabolism and excretion of phenols derived from olive products.

Journal: Functional Plant Science and Biotechnology **ISSN**: 1749-0472

Year: 2009

Volume: 3

Issue: Special Issue 1

Pages: 81-91
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.

URL: http://www.globalsciencebooks.info/JournalsSup/09FPSB_3_SI1.html, http://www.globalsciencebooks.info/JournalsSup/images/WebJournals/FPSB_3(SI1)Web.pdf, http://researchoutput.csu.edu.au/R/-?func=dbin-jump-full&object_id=20290&local_base=GEN01-CSU01,

Author Address: pprenzler@csu.edu.au
dryan@csu.edu.au
krobards@csu.edu.au

CRO Number: 20290

Absorption, Metabolism and Excretion of Phenols Derived from Olive Products

Megan Kendall[1], Marijka Batterham[2], Paul D. Prenzler[1], Danielle Ryan[1] and Kevin Robards[1]

[1] Charles Sturt University, School of Wine and Food Sciences, Wagga Wagga 2678, Australia

[2] Smart Food Centre, University of Wollongong, Northfields Ave Wollongong 2522, Australia

Mailing address

Kevin Robards

Charles Sturt University

School of Wine and Food Sciences

Locked Bag 588

Wagga Wagga 2678

AUSTRALIA

Phone 02 6933 2547

Fax 02 6933 2737

Email krobards@csu.edu.au

Running title: Nutrition of olive biophenols. Kendall *et al*.

Keywords: antioxidant, biophenol, health, leaf, olive

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, oleuroside, 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 up-take 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 bicompartmental 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 *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.

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 *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 tyrsosol 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 1 Studies of the absorption, metabolism and excretion of olive biophenols.

Compound	Absorption	Metabolism	Urinary excretion	Markers	Reference
Hydroxytyrosol, 1 - 4 mg ingested; tyrosol, 1.8 – 7.0 mg ingested	Postulated that tyrosol and hydroxytyrosol dose-dependently absorbed	Higher doses of phenols increased their rate of conjugation with glucuronide	Excreted in urine mainly as glucuronide; 20–28% and 30–60% ingested dose of tyrosol and hydroxytyrosol, respectively excreted. homovanillic alcohol excreted	Urinary excretion F_2-isoprostanes inversely related to phenol ingestion	Visioli *et al*. 2000; Visioli *et al*. 2002
Olive oil (tyrosol and hydroxytyrosol – measurement details not supplied)	Post-prandial absorption and incorporation into lipoproteins			LDL oxidizability and total plasma antioxidant capacity	Bonanome *et al*. 2000
Olive oil with different levels phenols			Hydroxytyrosol, homovanillic acid and alcohol excreted; hydrolysis step in method limits conclusions.		Caruso *et al*. 2001
Olive oil			Hydroxytyrosol and tyrosol excreted mainly as conjugates. Significant basal-level excretion of both compounds.		Miro-Casas *et al*. 2001
Oleuropein; polar supplement mainly hydroxytyrosol, tyrosol and oleuropein aglycone derivative; non-polar supplement mainly tyrosol and ligstroside aglycone derivative	Estimated 55-66% ingested phenols absorbed in small intestine not colon (structure and polarity regulate absorption)	Data supported absorption of intact phenols. Oleuropein degraded in gut and absorbed as hydroxytyrosol	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.		Vissers *et al*. 2002
Olive oil (hydroxytyrosol and 3-O-methylhydroxytyrosol measured; hydrolysis step.		Hydroxytyrosol present largely (ca 65%) as glucuronide conjugate with less than 2% free compound. Phenolic compounds are the subject of an extremely extensive first-pass intestinal/hepatic metabolism.	Urinary amounts of hydroxytyrosol and 3-*O*-methyl-hydroxytyrosol increased in response to virgin olive oil ingestion.		Miro-Casas *et al*. 2003a
Olive oil (hydroxytyrosol and tyrosol only measured)			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		Miro-Casas *et al*. 2003b

			same after both interventions. Method involved hydrolysis step, conjugates not measured.		
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

Al-Azzawie HF, Alhamdani MSS (2006) Hypoglycemic and antioxidant effect of oleuropein in alloxan-diabetic rabbits. *Life Sciences* **78**, 1371-1377

Andreadou I, Iliodromitis EK, Mikros E, Constantinou M, Agalias A, Magiatis P, Skaltsounis AL, Kamber E, Tsantili-Kakoulidou A, Kremastinos DT (2006) The olive constituent oleuropein exhibits anti-ischemic, antioxidative, and hypolipidemic effects in anesthetized rabbits. *Journal of Nutrition* **136**, 2213-2219

Andreadou I, Sigala F, Iliodromitis EK, Papaefthimiou M, Sigalas C, Aligiannis N, Savvari P, Gorgoulis V, Papalabros E, Kremastinos DT (2007) Acute doxorubicin cardiotoxicity is successfully treated with the phytochemical oleuropein through suppression of oxidative and nitrosative stress. *Journal of Molecular and Cellular Cardiology* **42**, 549-558

Bai C, Yan XJ, Takenaka M, Sekiya K, Nagata T (1998) Determination of synthetic hydroxytyrosol in rat plasma by GC-MS. *Journal of Agricultural and Food Chemistry* **46**, 3998-4001.

Bao J, Zhang DW, Zhang JZH, Huang PL, Huang PL, Lee-Huang S (2007) Computational study of bindings of olive leaf extract (OLE) to HIV-1 fusion protein gp41. *Febs Letters* **581**, 2737-2742.

Bazoti FN, Gikas E, Puel C, Coxam W, Tsarbopoulos A (2005) Development of a sensitive and specific solid phase extraction-gas chromatography-tandem mass spectrometry method for the determination of elenolic acid, hydroxytyrosol, and tyrosol in rat urine. *Journal of Agricultural and Food Chemistry* **53**, 6213-6221.

Beardsell D, Francis J, Ridley D, Robards K (2002) Health promoting constituents in plant derived edible oils. *Journal of Food Lipids* **9**, 1-34.

Benavente-Garcia O, Castillo J, Lorente J, Ortuno A, Del Rio JA (2000) Antioxidant activity of phenolics extracted from *Olea europaea* L-leaves. *Food Chemistry* **68**, 457-462.

Bendini A, Gomez-Caravaca AM, Cerretani L, Del Carlo M, Segura-Carretero A, Compagnone D, Cichelli A, Lercker G (2007) Evaluation of contribution of micro and macro components to oxidative stability on virgin oils obtained from olives characterized by different health quality. *Progress in Nutrition* **9**, 210-215.

Bonanome A, Pagnan A, Caruso D, Toia A, Xamin A, Fedeli E, Berra B, Zamburlini A, Ursini F, Galli G (2000) Evidence of postprandial absorption of olive oil phenols in humans. *Nutrition Metabolism and Cardiovascular Diseases* **10**, 111-120.

Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews* **56**, 317-333.

Briante R, Patumi M, Terenziani S, Bismuto E, Febbraio F, Nucci R (2002) *Olea* europaea L. leaf extract and derivatives: antioxidant properties. *Journal of Agricultural and Food Chemistry* **50**, 4934-4940.

Cabrini L, Barzanti V, Cipollone M, Fiorentini D, Grossi G, Tolomelli B, Zambonin L, Landi L (2001) Antioxidants and total peroxyl radical-trapping ability of olive and seed oils. *Journal of Agricultural and Food Chemistry* **49**, 6026-6032.

Cardoso SM, Guyot S, Marnet N, Lopes-DaSilva JA, Silva AMS, Renard C, Coimbra MA (2006) Identification of oleuropein oligomers in olive pulp and pomace. *Journal of the Science of Food and Agriculture* **86**, 1495-1502.

Caruso D, Berra B, Giavarini F, Cortesi N, Fedeli E, Galli G (1999) Effect of virgin olive oil phenolic compounds on *in vitro* oxidation of human low density lipoproteins. *Nutrition Metabolism and Cardiovascular Diseases* **9**, 102-107.

Caruso D, Visioli F, Patelli R, Galli C, Galli G (2001) Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism* **50**, 1426-1428.

Casas EM, Albadalejo MF, Planells MIC, Colomer MF, Raventos RML, Fornell RD (2001) Tyrosol bioavailability in humans after ingestion of virgin olive oil. *Clinical Chemistry* **47**, 341-343.

Coni E, Di Benedetto R, Di Pasquale M, Masella R, Modesti D, Mattei R, Carlini EA (2000) Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. *Lipids* **35**, 45-54.

Corona G, Tzounis X, Dessi MA, Deiana M, Debnam ES, Visioli F, Spencer JPE (2006) The fate of olive oil polyphenols in the gastrointestinal tract: implications of gastric and colonic microflora-dependent biotransformation. *Free Radical Research* **40**, 647-658.

Covas MI (2007) Olive oil and the cardiovascular system. *Pharmacological Research* **55**, 175-186.

Covas MI, Fito M, Lamuela-Raventos RM, Sebastia N, De La Torre-Boronat C, Marrugat J (2000) Virgin olive oil phenolic compounds: binding to human low density lipoprotein (LDL) and effect on LDL oxidation. *International Journal of Clinical Pharmacology Research* **20**, 49-54.

Covas MI, Miro-Casas E, Fito M, Farre-Albadalejo M, Gimeno E, Marrugat J, De La Torre R (2003) Bioavailability of tyrosol, an antioxidant phenolic compound present in wine and olive oil, in humans. *Drugs Under Experimental and Clinical Research* **29**, 203-206.

Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJF, Kiesewetter H, Gaddi A, De La Torre R, Mursu J, Baumler H, Nascetti S, Salonen JT, Fito M, Virtanen J, Marrugat J (2006a) The effect of polyphenols in olive oil on heart disease risk factors - a randomized trial. *Annals of Internal Medicine* **145**, 333-341.

Covas MI, Ruiz-Gutierrez V, De La Torre R, Kafatos A, Lamuela-Raventos RM, Osada J, Owen RW, Visioli F (2006b) Minor components of olive oil: evidence to date of health benefits in humans. *Nutrition Reviews* **64**, S20-S30

Craig W, Beck L (1999) Phytochemicals: health protective effects. *Canadian Journal of Dietetic Practice and Research* **60**, 78-84.

D'Angelo S, Manna C, Migliardi V, Mazzoni O, Morrica P, Capasso G, Pontoni G, Galletti P, Zappia V (2001) Pharmacokinetics and metabolism of hydroxytyrosol, a natural antioxidant from olive oil. *Drug Metabolism and Disposition* **29**, 1492-1498.

Day AJ, Dupont MS, Ridley S, Rhodes M, Rhodes MJC, Morgan MRA, Williamson G (1998) Deglycosylation of flavonoid and isoflavonoid glycosides by human small intestine and liver beta-glucosidase activity. *Febs Letters* **436**, 71-75.

De La Torre-Carbot K, Chavez-Servin JL, Jauregui O, Castellote AI, Lamuela-Raventos RM, Fito M, Covas MI, Munoz-Aguayo D, Lopez-Sabater MC (2007) Presence of virgin olive oil phenolic metabolites in human low density lipoprotein fraction: determination by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Analytica Chimica Acta* **583**, 402-410.

De La Torre-Carbot K, Jauregui O, Castellote AI, Lamuela-Raventos RM, Covas MI, Casals I, Lopez-Sabater MC (2006) Rapid high-performance liquid chromatography-electro spray ionization tandem mass spectrometry method for qualitative and quantitative analysis of virgin olive oil phenolic metabolites in human low-density lipoproteins. *Journal of Chromatography A* **1116**, 69-75.

De Leonardis A, Aretini A, Alfano G, Macciola V, Ranalli G (2008) Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea europaea* L.) and evaluation of its antioxidant properties and bioactivity. *European Food Research and Technology* **226**, 653-659.

Del Boccio P, Di Deo A, De Curtis A, Celli N, Iacoviello L, Rotilio D (2003) Liquid chromatography-tandem mass spectrometry analysis of oleuropein and its metabolite hydroxytyrosol in rat plasma and urine after oral administration. *Journal of Chromatography B* **785**, 47-56.

Di Donna L, Mazzotti F, Salerno R, Tagarelli A, Taverna D, Sindona G (2007) Characterization of new phenolic compounds from leaves of *Olea europaea* L. by high-resolution tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **21**, 3653-3657.

Edgecombe SC, Stretch GL, Hayball PJ (2000) Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. *Journal of Nutrition* **130**, 2996-3002.

Fernandez-Bolanos JG, Lopez O, Fernandez-Bolanos J, Rodriguez-Gutierrez G (2008) Hydroxytyrosol and derivatives: isolation, synthesis, and biological properties. *Current Organic Chemistry* **12**, 442-463.

Ferreira I, Barros L, Soares ME, Bastos ML, Pereira JA (2007) antioxidant activity and phenolic contents of olea europaea l. leaves sprayed with different copper formulations. *Food Chemistry* **103**, 188-195.

Fito M, Covas MI, Lamuela-Raventos RM, Vila J, Torrents J, De La Torre C, Marrugat J (2000) Protective effect of olive oil and its phenolic compounds against low density lipoprotein oxidation. *Lipids* **35**, 633-638.

Franconi F, Coinu R, Carta S, Urgeghe PP, Ieri F, Mulinacci N, Romani A (2006) Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. *Journal of Agricultural and Food Chemistry* **54**, 3121-3125.

Frankel EN, Finley JW (2008) How to standardize the multiplicity of methods to evaluate natural antioxidants. *Journal of Agricultural and Food Chemistry* **56**, 4901-4908.

Fraser GE (1994) Diet and coronary heart-disease - beyond dietary fats and low-density-lipoprotein cholesterol. *American Journal of Clinical Nutrition* **59**, S1117-S1123

Giamarellos-Bourboulis EJ, Geladopoulos T, Chrisofos M, Koutoukas P, Vassiliadis J, Alexandrou I, Tsaganos T, Sabracos L, Karagianni V, Pelekanou E, Tzepi I, Kranidioti H, Koussoulas V, Giamarellou H (2006) Oleuropein: a novel immunomodulator conferring prolonged survival in experimental sepsis by pseudomonas aeruginosa. *Shock* **26**, 410-416.

Gikas E, Bazoti FN, Tarbopoulos A (2007) Conformation of oleuropein, the major bioactive compound of *Olea europea*. *Journal of Molecular Structure-Theochem* **821**, 125-132.

Gimeno E, Fito M, Lamuela-Raventos RM, Castellote AI, Covas M, Farre M, De La Torre-Boronat MC, Lopez-Sabater MC (2002) Effect of ingestion of virgin olive oil on human low-density lipoprotein composition . *European Journal of Clinical Nutrition* **56**, 114-120.

Gorelik S, Ligumsky M, Kohen R, Kanner J (2008a) A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products. *Faseb Journal* **22**, 41-46.

Gorelik S, Ligumsky M, Kohen R, Kanner J (2008b) The stomach as a "bioreactor":

when red meat meets red wine. *Journal of Agricultural and Food Chemistry* **56**, 5002-5007.

Halliwell B (1999) Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. *Nutrition Reviews* **57**, 104-113.

Halliwell B, Whiteman M (2004) Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? *British Journal of Pharmacology* **142**, 231-255.

Hamdi HK, Castellon R (2005) Oleuropein, a non-toxic olive iridoid, is an anti-tumor agent and cytoskeleton disruptor. *Biochemical and Biophysical Research Communications* **334**, 769-778.

Hermans N, Cos P, Maes L, De Bruyne T, Berghe DV, Vlietinck AJ, Pieters L (2007) Challenges and pitfalls in antioxidant research. *Current Medicinal Chemistry* **14**, 417-430.

Hollman PCH, Devries JHM, Vanleeuwen SD, Mengelers MJB, Katan MB (1995) Absorption of dietary quercetin glycosides and quercetin in healthy ileostomy volunteers. *American Journal of Clinical Nutrition* **62**, 1276-1282.

Hu M (2007) Commentary: bioavailability of flavonoids and polyphenols: call to arms. *Molecular Pharmaceutics* **4**, 803-806.

Hwang ES, Kim GH (2007) Biomarkers for oxidative stress status of DNA, lipids, and proteins *in vitro* and *in vivo* cancer research. *Toxicology* **229**, 1-10.

Ito H, Gonthier MP, Manach C, Morand C, Mennen L, Remesy C, Scalbert A (2005) Polyphenol levels in human urine after intake of six different polyphenol-rich beverages. *British Journal of Nutrition* **94**, 500-509.

Japon-Lujan R, De Castro L (2006) Superheated liquid extraction of oleuropein and related biophenols from olive leaves. *Journal of Chromatography A* **1136**, 185-191.

Japon-Lujan R, De Castro MDL (2007) Small branches of olive tree: a source of biophenols complementary to olive leaves. *Journal of Agricultural and Food Chemistry* **55**, 4584-4588.

Japon-Lujan R, De Castro MDL (2008) Liquid-liquid extraction for the enrichment of edible oils with phenols from olive leaf extracts. *Journal of Agricultural and Food Chemistry* **56**, 2505-2511.

Japon-Lujan R, Ruiz-Jimenez J, De Castro MDL (2006) Discrimination and classification of olive tree varieties and cultivation zones by biophenol contents. *Journal of Agricultural and Food Chemistry* **54**, 9706-9712.

Jenkins DJA, Kendall CWC, Ransom TPP (1998) Dietary fiber, the evolution of the human diet and coronary heart disease. *Nutrition Research* **18**, 633-652.

Kanner J, Lapidot T (2001) The stomach as a bioreactor: dietary lipid peroxidation in the gastric fluid and the effects of plant-derived antioxidants. *Free Radical Biology and Medicine* **31**, 1388-1395.

Korukluoglu M, Sahan Y, Yigit A, Karakas R (2006) Antifungal activity of olive leaf (*Olea europaea* L.) extracts from the trilye region of turkey. *Annals of Microbiology* **56**, 359-362.

Lavelli V (2007) Degradation kinetic of the antioxidant activity of extra virgin olive oil during storage. *Progress in Nutrition* **9**, 204-209.

Lee-Huang S, Huang PL, Zhang DW, Lee JW, Bao J, Sun YT, Chang YT, Zhang J, Huang PL (2007a) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: part I. Integrase inhibition. *Biochemical and Biophysical Research Communications* **354**, 872-878.

Lee-Huang S, Huang PL, Zhang DW, Lee JW, Bao J, Sun YT, Chang YT, Zhang J, Huang PL (2007b) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: part II. integrase inhibition. *Biochemical and Biophysical Research Communications* **354**, 879-884.

Lee-Huang S, Huang PL, Zhang DW, Lee JW, Bao J, Sun YT, Chang YT, Zhang J, Huang PL (2007c) Discovery of small-molecule HIV-1 fusion and integrase inhibitors oleuropein and hydroxytyrosol: part 1. Integrase inhibition (vol 354, pg 872, 2007). *Biochemical and Biophysical Research Communications* **356**, 1068

Ligumsky M, Gorelik S, Kanner J, Kohen R (2008) Red wine polyphenols reduce postprandial lipid peroxidation end products absorption. *Gastroenterology* **134**, A584

Lipworth L, Martinez ME, Angell J, Hsieh CC, Trichopoulos D (1997) Olive oil and human cancer: an assessment of the evidence. *Preventive Medicine* **26**, 181-190.

Machowetz A, Poulsen HE, Gruendel S, Weimann A, Fito M, Marrugat J, De La Torre R, Salonen JT, Nyyssonen K, Mursu J, Nascetti S, Gaddi A, Kiesewetter H, Baumler H, Selmi H, Kaikkonen J, Zunft HJF, Covas MI, Koebnick C (2007) Effect of olive oils on biomarkers of oxidative DNA stress in northern and southern Europeans. *Faseb Journal* **21**, 45-52.

Malik NSA, Bradford JM (2006) Changes in oleuropein levels during differentiation and development of floral buds in 'arbequina' olives. *Scientia Horticulturae* **110**, 274-278.

Malik NSA, Bradford JM (2008) Recovery and stability of oleuropein and other phenolic compounds during extraction and processing of olive (*Olea europaea* L.) leaves. *Journal of Food Agriculture & Environment* **6**, 8-13.

Manach C, Donovan JL (2004) Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radical Research* **38**, 771-785.

Manach C, Williamson G, Morand C, Scalbert A, Remesy C (2005) Bioavailability and bioefficacy of polyphenols in humans. I. review of 97 bioavailability studies. *American Journal of Clinical Nutrition* **81**, 230S-242S.

Manna C, Galletti P, Cucciolla V, Moltedo O, Leone A, Zappia V (1997) The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *Journal of Nutrition* **127**, 286-292.

Manna C, Galletti P, Maisto G, Cucciolla V, D'Angelo S, Zappia V (2000) Transport mechanism and metabolism of olive oil hydroxytyrosol in Caco-2 cells. *Febs Letters* **470**, 341-344.

Manna C, Migliardi V, Golino P, Scognamiglio A, Galletti P, Chiariello M, Zappia V (2004) Oleuropein prevents oxidative myocardial injury induced by ischemia and reperfusion. *Journal of Nutritional Biochemistry* **15**, 461-466.

Martinez-Gonzalez MA, Fernandez-Jarne E, Martinez-Losa E, Prado-Santamaria M, Brugarolas-Brufau C, Serrano-Martinez M (2002) Role of fibre and fruit in the mediterranean diet to protect against myocardial infarction: a case-control study in Spain. *European Journal of Clinical Nutrition* **56**, 715-722.

Mazziotti A, Mazzotti F, Pantusa M, Sportelli L, Sindona G (2006) Pro-oxidant activity of oleuropein determined *in vitro* by electron spin resonance spin-trapping methodology. *Journal of Agricultural and Food Chemistry* **54**, 7444-7449.

Medina E, De Castro A, Romero C, Brenes M (2006) Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *Journal of Agricultural and Food Chemistry* **54**, 4954-4961.

Miro-Casas E, Albaladejo MF, Covas MI, Rodriguez JO, Colomer EM, Raventos RML, De La Torre R (2001) Capillary gas chromatography-mass spectrometry quantitative determination of hydroxytyrosol and tyrosol in human urine after olive oil intake. *Analytical Biochemistry* **294**, 63-72.

Miro-Casas E, Covas MI, Farre M, Fito M, Ortuno J, Weinbrenner T, Roset P, De La Torre R (2003a) Hydroxytyrosol disposition in humans. *Clinical Chemistry* **49**, 945-952.

Miro-Casas E, Covas MI, Fito M, Farre-Albadalejo M, Marrugat J, De La Torre R (2003b) Tyrosol and hydroxytyrosol are absorbed from moderate and sustained doses of virgin olive oil in humans. *European Journal of Clinical Nutrition* **57**, 186-190.

Morton LW, Caccetta RA, Puddey IB, Croft KD (2000) Chemistry and biological

effects of dietary phenolic compounds: relevance to cardiovascular disease. *Clinical and Experimental Pharmacology and Physiology* **27**, 152-159.

Obied HK, Allen MS, Bedgood DR, Prenzler PD, Robards K, Stockmann R (2005) Bioactivity and analysis of biophenols recovered from olive mill waste. *Journal of Agricultural and Food Chemistry* **53**, 823-837.

Ortega-Garcia F, Blanco S, Peinad MA, Peragon J (2008) Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'picual' trees during fruit ripening. *Tree Physiology* **28**, 45-54.

Owen RW, Giacosa A, Hull WE, Haubner R, Spiegelhalder B, Bartsch H (2000a) The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *European Journal of Cancer* **36**, 1235-1247.

Owen RW, Mier W, Giacosa A, Hull WE, Spiegelhalder B, Bartsch H (2000b) Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food and Chemical Toxicology* **38**, 647-659.

Paiva-Martins F, Gordon MH (2001) Isolation and characterization of the antioxidant component 3,4-dihydroxyphenylethyl 4-formyl-3-formylmethyl-4-hexenoate from olive (*Olea europaea*) leaves. *Journal of Agricultural and Food Chemistry* **49**, 4214-4219.

Papadopoulos G, Boskou D (1991) Antioxidant effect of natural phenols on olive oil. *Journal of the American Oil Chemists Society* **68**, 669-671.

Parr AJ, Bolwell GP (2000) Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. *Journal of the Science of Food and Agriculture* **80**, 985-1012.

Petroni A, Blasevich M, Salami M, Papini N, Montedoro GF, Galli C (1995) Inhibition of platelet-aggregation and eicosanoid production by phenolic components of olive oil. *Thrombosis Research* **78**, 151-160.

Puel C, Mathey J, Agalias A, Kati-Coulibaly S, Mardon J, Obled C, Davicco MJ, Lebecque P, Horcajada MN, Skaltsounis AL, Coxam V (2006) Dose-response study of effect of oleuropein, an olive oil polyphenol, in an ovariectomy/inflammation experimental model of bone loss in the rat. *Clinical Nutrition* **25**, 859-868.

Puel C, Quintin A, Agalias A, Mathey J, Obled C, Mazur A, Davicco MJ, Lebecque P, Skaltsounis AL, Coxam V (2004) Olive oil and its main phenolic micronutrient (oleuropein) prevent inflammation-induced bone loss in the ovariectomised rat. *British Journal of Nutrition* **92**, 119-127.

Rabovsky A, Cuomo J, Eich N (2006) Measurement of plasma antioxidant reserve after supplementation with various antioxidants in healthy subjects. *Clinica Chimica Acta* **371**, 55-60.

Ranalli A, Contento S, Lucera L, Di Febo M, Marchegiani D, Di Fonzo V (2006) Factors affecting the contents of iridoid oleuropein in olive leaves (*Olea europaea* L.). *Journal of Agricultural and Food Chemistry* **54**, 434-440.

Reaven PD, Witztum JL (1995) The role of oxidation of LDL in atherogenesis. *Endocrinologist* **5**, 44-54.

Rechner AR, Kuhnle G, Bremner P, Hubbard GP, Moore KP, Rice-Evans CA (2002) The metabolic fate of dietary polyphenols in humans. *Free Radical Biology and Medicine* **33**, 220-235.

Rietjens SJ, Bast A, Haenen Grmm (2007) New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. *Journal of Agricultural and Food Chemistry* **55**, 7609-7614.

Romani A, Lapucci C, Cantini C, Ieri F, Mulinacci N, Visioli F (2007) Evolution of minor polar compounds and antioxidant capacity during storage of bottled extra virgin olive oil. *Journal of Agricultural and Food Chemistry* **55**, 1315-1320.

Ruiz-Gutierrez V, Juan ME, Cert A, Planas JM (2000) Determination of hydroxytyrosol in plasma by HPLC. *Analytical Chemistry* **72**, 4458-4461.

Ruiz-Gutiérrez V, Muriana FJG, Maestro R, Graciani E (1995) Oleuropein on lipid and fatty-acid composition of rat-heart. *Nutrition Research* **15**, 37-51.

Ryan D, Lawrence H, Prenzler PD, Antolovich M, Robards K (2001) Recovery of phenolic compounds from *Olea europaea. Analytica Chimica Acta* **445**, 67-77.

Saija A, Uccella N (2001) Olive biophenols: functional effects on human wellbeing. *Trends in Food Science & Technology* **11**, 357-363.

Salami M, Galli C, Deangelis L, Visioli F (1995) Formation of f-2-isoprostanes in oxidized low-density-lipoprotein - inhibitory effect of hydroxytyrosol. *Pharmacological Research* **31**, 275-279.

Salta FN, Mylona A, Chiou A, Boskou G, Andrikopoulos NK (2007) Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. *Food Science and Technology International* **13**, 413-421.

Scalbert A, Manach C, Morand C, Remesy C (2004) Bioavailability of dietary polyphenols is a key issue to assess their impact on physiological functions. *Free Radical Biology and Medicine* **36**, S27

Scalbert A, Morand C, Manach C, Remesy C (2002) Absorption and metabolism of polyphenols in the gut and impact on health. *Biomedicine & Pharmacotherapy* **56**, 276-282.

Scalbert A, Williamson G (2000) Dietary intake and bioavailability of polyphenols.

Journal of Nutrition **130**, 2073S-2085S.

Schrezenmeir J, Korhonen H, Williams C, Gill HS, Shah N (2000) Beneficial natural bioactive substances in milk and colostrum - occurrence, biochemical and technological characteristics of bioactive substances - physiological effects and potential health benefits - foreword. *British Journal of Nutrition* **84**, S1

Serra-Majem L, Roman B, Estruch R (2006) Scientific evidence of interventions using the mediterranean diet: a systematic review. *Nutrition Reviews* **64**, S27-S47

Silberberg M, Morand C, Mathevon T, Besson C, Manach C, Scalbert A, Remesy C (2006) The bioavailability of polyphenols is highly governed by the capacity of the intestine and of the liver to secrete conjugated metabolites. *European Journal of Nutrition* **45**, 88-96.

Silva S, Gomes L, Leitao F, Coelho AV, Boas LV (2006) Phenolic compounds and antioxidant activity of *Olea europaea* L. fruits and leaves. *Food Science and Technology International* **12**, 385-395.

Simopoulos AP (2001) The Mediterranean diets: what is so special about the diet of Greece? the scientific evidence. *Journal of Nutrition* **131**, 3065S-3073S.

Somova LO, Nadar A, Rammanan P, Shode FO (2003) Cardiovascular, antihyperlipidemic and antioxidant effects of oleanolic and ursolic acids in experimental hypertension. *Phytomedicine* **10**, 115-121.

Spencer JPE, Mohsen MMA, Minihane AM, Mathers JC (2008) Biomarkers of the intake of dietary polyphenols: strengths, limitations and application in nutrition research. *British Journal of Nutrition* **99**, 12-22.

Speroni E, Guerra MC, Minghetti A, Crespi-Perellino N, Pasini P, Piazza F, Roda A (1998) Oleuropein evaluated *in vitro* and *in vivo* as an antioxidant. *Phytotherapy Research* **12**, S98-S100

Stretch GL, Nation RL, Evans AM, Milne RW (1999) Organ perfusion techniques in drug development. *Drug Development Research* **46**, 292-301.

Tripoli E, Giammanco M, Tabacchi G, Di Majo D, Giammanco S, La Guardia M (2005) The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutrition Research Reviews* **18**, 98-112.

Tuck KL, Freeman MP, Hayball PJ, Stretch GL, Stupans I (2001) The *in vivo* fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *Journal of Nutrition* **131**, 1993-1996.

Tuck KL, Hayball PJ (2002) Major phenolic compounds in olive oil: metabolism and health effects. *Journal of Nutritional Biochemistry* **13**, 636-644.

Tzika ED, Papadimitriou V, Sotiroudis TG, Xenakis A (2008) Oxidation of oleuropein studied by EPR and spectrophotometry. *European Journal of Lipid Science and Technology* **110**, 149-157.

Visioli F, Bellomo G, Galli C (1998) Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications* **247**, 60-64.

Visioli F, Caruso D, Galli C, Viappiani S, Galli G, Sala A (2000) Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochemical and Biophysical Research Communications* **278**, 797-799.

Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, Sirtori C, Galli C (2005) Virgin olive oil study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *European Journal of Nutrition* **44**, 121-127.

Visioli F, Galli C (1994) Oleuropein protects low-density-lipoprotein from oxidation. *Life Sciences* **55**, 1965-1971.

Visioli F, Galli C (1998) Olive oil phenols and their potential effects on human health. *Journal of Agricultural and Food Chemistry* **46**, 4292-4296.

Visioli F, Galli C (2002) Biological properties of olive oil phytochemicals. *Critical Reviews in Food Science and Nutrition* **42**, 209-221.

Visioli F, Galli C, Bornet F, Mattei A, Patelli R, Galli G, Caruso D (2000) Olive oil phenolics are dose-dependently absorbed in humans. *Febs Letters* **468**, 159-160.

Visioli F, Galli C, Galli G, Caruso D (2002) Biological activities and metabolic fate of olive oil phenols. *European Journal of Lipid Science and Technology* **104**, 677-684.

Visioli F, Galli C, Grande S, Colonnelli K, Patelli C, Galli G, Caruso D (2003) Hydroxytyrosol excretion differs between rats and humans and depends on the vehicle of administration. *Journal of Nutrition* **133**, 2612-2615.

Visioli F, Poli A, Galli C (2002) Antioxidant and other biological activities of phenols from olives and olive oil. *Medicinal Research Reviews* **22**, 65-75.

Visioli F, Vinceri FF, Galli C (1995) Waste-waters from olive oil production are rich in natural antioxidants. *Experientia* **51**, 32-34.

Vissers MN, Zock PL, Katan MB (2004) Bioavailability and antioxidant effects of olive oil phenols in humans: a review. *European Journal of Clinical Nutrition* **58**, 955-965.

Vissers MN, Zock PL, Leenen R, Roodenburg AJC, Van Putte Kpam, Katan MB (2001) Effect of consumption of phenols from olives and extra virgin olive oil

on LDL oxidizability in healthy humans. *Free Radical Research* **35**, 619-629.

Vissers MN, Zock PL, Roodenburg AJC, Leenen R, Katan MB (2002) Olive oil phenols are absorbed in humans. *Journal of Nutrition* **132**, 409-417.

Vissers MN, Zock PL, Wiseman SA, Meyboom S, Katan MB (2001) Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. *European Journal of Clinical Nutrition* **55**, 334-341.

Walle T, Browning AM, Steed LL, Reed SG, Walle UK (2005) Flavonoid glucosides are hydrolyzed and thus activated in the oral cavity in humans. *Journal of Nutrition* **135**, 48-52.

Walle T, Walle UK, Halushka PV (2001) Carbon dioxide is the major metabolite of quercetin in humans. *Journal of Nutrition* **131**, 2648-2652.

Waterman E, Lockwood B (2007) Active components and clinical applications of olive oil. *Alternative Medicine Review* **12**, 331-342.

Weinbrenner T, Fito M, Farre AM, Saez GT, Rijken P, Tormos C, Coolen S, De la Torre R, Covas MI (2004) Bioavailability of phenolic compounds from olive oil and oxidative/antioxidant status at postprandial state in healthy humans. *Drugs under Experimental and Clinical Research* **30**, 207-212.

Williamson G, Manach C (2005) Bioavailability and bioefficacy of polyphenols in humans. II. review of 93 intervention studies. *American Journal of Clinical Nutrition* **81**, 243S-255S.

Witztum JL (1994) The oxidation hypothesis of atherosclerosis. *Lancet* **344**, 793-795.

Yang DP, Kong DX, Zhang HY (2007) Multiple pharmacological effects of olive oil phenols. *Food Chemistry* **104**, 1269-1271.

Zarzuelo A, Duarte J, Jimenez J, Gonzalez M, Utrilla MP (1991) Vasodilator effect of olive leaf. *Planta Medica* **57**, 417-419.