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Major phenolic compounds in olive oil: metabolism and health effects

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Abstract

It has been postulated that the components in olive oil in the Mediterranean diet, a diet which is largely vegetarian in nature, can contribute to the lower incidence of coronary heart disease and prostate and colon cancers. The Mediterranean diet includes the consumption of large amounts of olive oil. Olive oil is a source of at least 30 phenolic compounds. The major phenolic compounds in olive oil are oleuropein, hydroxytyrosol and tyrosol. Recently there has been a surge in the number of publications that has investigated their biological properties. The phenolic compounds present in olive oil are strong antioxidants and radical scavengers. Olive "waste water" also possesses compounds which are strong antioxidant and radical scavengers. Typically, hydroxytyrosol is a superior antioxidant and radical scavenger to oleuropein and tyrosol. Hydroxytyrosol and oleuropein have antimicrobial activity against ATTC bacterial strains and clinical bacterial strains. Recent syntheses of labeled and unlabelled hydroxytyrosol coupled with superior analytical techniques have enabled its absorption and metabolism to be studied. It has recently been found that hydroxytyosol is renally excreted unchanged and as the following metabolites as its glucuronide conjugate, sulfate conjugate, homovanillic acid, homovanillic alcohol, 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylacetaldehyde. Studies with tyrosol have shown that it is excreted unchanged and as its conjugates. This review summarizes the antioxidant abilities; the scavenging abilities and the biological fates of hydroxytyrosol, oleuropein and tyrosol which have been published in recent years.

Keywords: Olive oil; Mediterranean diet; Hydroxytyrosol; Tyrosol; Oleuropein; Phenolic compounds.

1. Introduction

In the last couple of years the number of reports describing the beneficial properties of olive oil has dramatically increased. Recent data has suggested that the components in olive oil may have more health benefits that previously thought and consequently there have been numerous experiments which have investigated the fate of the constituents in olive oil. It has been speculated that consumption of olive oil contributes to the lower incidence of coronary heart disease and some cancers. It has been postulated that the lower incidence of coronary heart disease and prostate and colon cancers in Greece, Italy and Spain is due to the Mediterranean diet. The Mediterranean diet is largely vegetarian in nature and the consumption of olive oil is the principal source of fat. The amount of vegetable fat obtained via olive oil is 71, 42 and 37% in that in Greece, Italy and Spain respectively [1]. The amount of olive oil consumed in Greece is 18 kg per year per capita, in Italy 13 kg per year per capita and in Spain 11 kg per year per capita [2].

The composition of olive oil is primarily triacylglycerols and ~0.5%-1.0% nonglyceridic constituents [3]. Olive oil is also a source of at least 30 phenolic compounds [4–8], many of which contribute to the resistance of olive oil to oxidative rancidity [9]. It has been found that a linear relationship exists between the phenolic content and oxidative stability of extra-virgin olive oil [10]. The phenolic content of olive oil depends on a number of factors but it is mainly dependent on the production and storage of the oil [11]. The total phenol content is higher in extra-virgin olive oil than in refined virgin olive oil [12].

2. Chemistry of phenolics

2.1. Phenolic content in olive oil

The total phenol content of olive oil has been reported numerous times in the literature, however there are incon-

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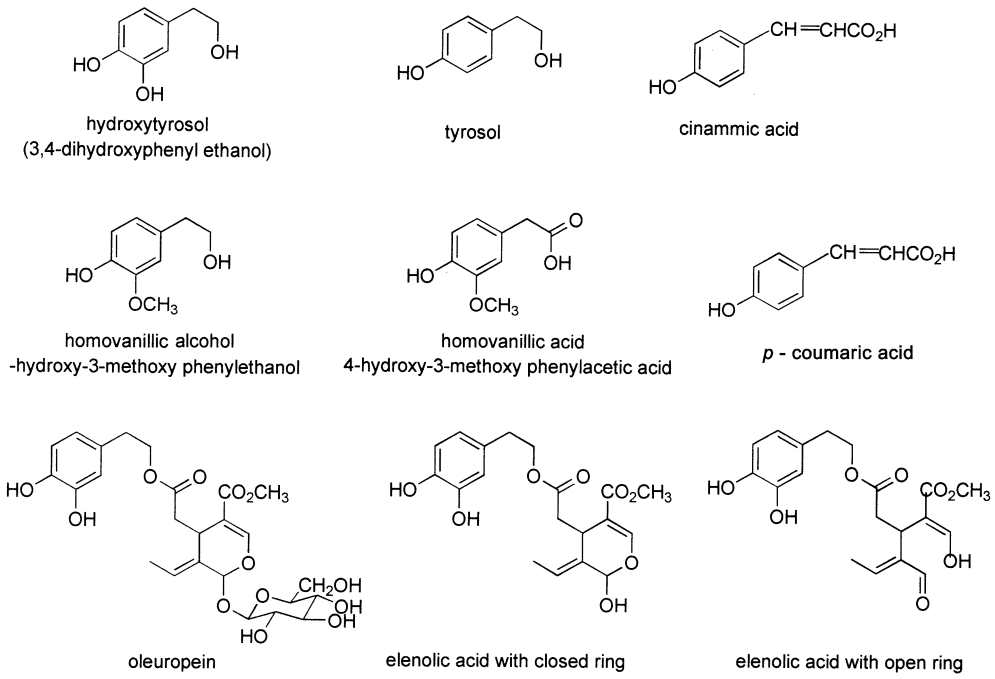

Fig. 1. The major constituents of olive oil.

sistencies with the concentrations obtained. The source of this discrepancy could be due to inaccuracy of the two methods commonly used to determine total phenol content. These methods are the Folin-Ciocalteau reagent (which is not specific for phenols) followed by analysis by UV and extraction of the oil and analysis by HPLC (which is limited by the extraction procedure and the complexity of the phenolic fraction). The total phenol content in olive oil has been reported to vary between 800 mg/kg and 1g/kg [13], to be over 500 mg/kg [14], to be 232 ± 15 mg/kg in extra-virgin olive oil and 62 ± 12 mg/kg in refined olive oil [12], it has also been reported that the concentration of total phenol content varies from 100 to 800 mg/kg [15]. Using an average total phenol content of 500 mg/kg, an estimate of the amount of olive oil phenolics consumed per person per year in Greece is 9 g, in Italy 7.5 g and in Spain 5.5 g.

The major phenolic compounds in olive oil are shown in Fig. 1. The three phenolic compounds in highest concentration in olive oil are the glycoside oleuropein, hydroxytyrosol (3,4-dihydroxyphenyl ethanol) and tyrosol. These three compounds are related structurally. Hydroxytyrosol and tyrosol are structurally identical except that hydroxytyrosol posses an extra hydroxy group in the *meta* position. Oleuropein is an ester which consists of hydroxytyrosol and elenolic acid. Oleuropein is the major phenolic compound in olive fruit, which can be as much as 14% in dried fruit, hydroxytyrosol is the major phenolic component in olive oil [16]. As the olive fruit matures the concentration of oleuropein decreases and hydroxytyrosol, a hydrolysis product of oleuropein increases [17,18].

As with the total phenol content of olive oil the content of oleuropein, hydroxytyrosol and tyrosol in olive oil has varied in the literature. The concentration of hydroxytyrosol in olive oil has been reported to be 1.4–5.6 mg/L [14]; 1.63 ± 0.25 mg/kg [19]; and 14.42 ± 3.01 mg/kg in extra-virgin olive oil and 1.74 ± 0.84 mg/kg in refined virgin oil [12]. The concentration of tyrosol in olive oil has been reported to be 4.69 ± 0.77 mg/kg [19]; and 27.45 ± 4.05 mg/kg in extra-virgin olive oil and 2.98 ± 1.33 mg/kg in refined virgin oil [12]. The concentration of oleuropein has been reported to be 2.3–9.0 mg/L [14]; and 2.04 ± 0.78 mg/kg, in extra virgin olive oil and in the one of the samples the concentration of oleuropein aglycone was 18.64 ± 3.36 mg/kg [19] A correlation between the concentration of hydroxytyrosol and the stability of oil exists, however the same does not apply for tyrosol [20,21].

Despite the myriad papers published on the beneficial effects of olive oil in the past decades only recently, in the past five years, have the fate in the body of the phenolic compounds present in olive oil been investigated. This review focuses on the recent publications on the antioxidant phenolic constituents, oleuropein, hydroxytyrosol and tyrosol, present in olive oil and their recent findings on their metabolism and health effects.

2.2. Syntheses of hydroxytyrosol

Until recently there have been limited reports on the absorption and disposition on hydroxytyrosol. This lack of data is probably because hydroxytyrosol is easily oxidized and has only been recently commercially available. It is important that it can be synthesized easily so that investi-

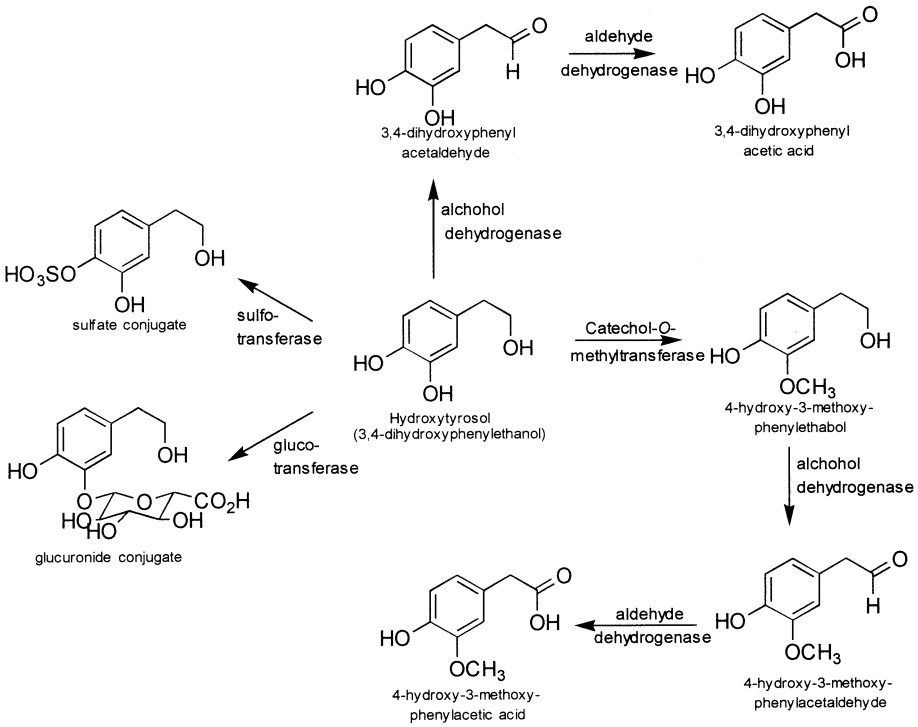

Fig. 2. Postulation of the enzymatic pathways for the metabolites of hydroxytyrosol in vivo.

gation of its biological properties can more readily occur. There are several methods for the synthesis of hydroxytyrosol and recently there have been several publications for the synthesis of the labeled compound.

Hydroxytyrosol can be synthesized from (3,4-dihydroxyphenyl)acetic acid by several routes; by direct reduction with aluminum hydride [22], by reduction with (trimethylsilyl)diazomethane and sodium borohydride [23], and by reduction of the acid with tetrabutylammonium boronate [24]. The methyl ester of (3,4-dihydroxyphenyl)acetic acid can be reduced with lithium aluminum hydride to give hydroxytyrosol [25]. Recently, hydroxytyrosol has been enzymatically synthesized using mushroom tyrosinase [26]. This procedure is environmentally friendly and is adaptable to industrial processes. The commercial mushroom tyrosinase is expensive, however it can be collected and reused. Labeled (deuterium or tritium) hydroxytyrosol has been synthesized by two routes [24,27]. The label is incorporated in the aromatic ring after a heterogeneous acid-catalyzed H/D or H/T exchange with either Amberlyst 15 or Nafion with hydroxytyrosol [27]. Alternatively, reduction of (3,4-dihydroxyphenyl)acetic acid with labeled tetrabutylammonium boronate results in incorporation of a deuterium label at the C2 position [24]. Labeled (^{14}C) hydroxytyrosol is commercially available (reported in [28]). This recent availability of labeled hydroxytyrosol has enabled several studies on the absorption, metabolism and disposition of hydroxytyrosol which will be discussed later.

2.3. Analytical techniques for quantification of oleuropein, hydroxytyrosol and tyrosol in plasma

There have been several studies which have quantified the amount of oleuropein, hydroxytyrosol, or tyrosol in plasma after oral dosing to humans or rats. There have been two studies which have used plasma samples spiked with hydroxytyrosol to validate analytical methods [19,29]. A HPLC method has been developed for the identification and quantification of hydroxytyrosol in human plasma. This procedure avoided the successive use of SPE cartridges and plasma samples were analyzed by HPLC analysis after their simple concentration [29].

An alternative experiment examined the LDL oxidazability of oleuropein in rabbits. In this experiment the plasma samples were extracted using SPE cartridges, after evaporation of the solvent the dry residue was reconstituted and analyzed by HPLC [19].

Their have been two reports of the urinary levels of hydroxytyrosol by GC-MS after extraction and formation of the trimethylsilyl derivates of the plasma samples [23,30].

3. Beneficial health benefits of phenolic constituents in olive oil

3.1. The beneficial effects of the phenolic constituents in olive oil

There have been many reports on the lower incidence of cancer in animals and humans after consumption of olive

oil. A review, by Lipworth et al., summarizes the association of olive oil intake with cancer risk in humans [19]. It was concluded in this review that olive oil does not have the cancer-promoting potential of other fat types. However, additional studies will be required to confirm this hypothesis.

Numerous studies have shown that these phenols are potent inhibitors of LDL oxidation in vitro [7,8]. The in vivo oxidation of LDL is linked to the formation of atherosclerotic plaques, which are postulated to contribute to the development of coronary heart disease. Olive oil phenols have also been beneficially linked to processes that contributes to the pathogenesis of heart disease and cancer [5].

In particular hydroxytyrosol, one of the major phenolic constituent in olive oil, has been reported to alone reduce the risk of coronary heart disease and atheroscelosis [31,32]. It has also been postulated that hydroxytyrosol inhibits arachidonic acid lipoxygenase [33] or inhibits platelet aggregation [34,35]. It is presumed that hydroxytyrosol penetrates in cell membranes and consequently can inhibit the production of leukotriene B4 (LTB4) effectively from endogenous arachidonic acid [4].

Oleuropein inhibits androstenedione 6β-hydroxylase activity, a CYP3A marker in human liver microsomes [36] and oleuropein, but not the structurally similar compounds hydroxytyrosol and secologanin, was found to be a mechanism-based inhibitor of androstenedione 6β-hydroxylase activity [37].

3.2. Antioxidant effects of phenolic compounds

Due to the recent interest in the phenolic components present in olive oil there have been numerous studies on their antioxidant properties. It is commonly known that compounds which share an orthodiphenolic (catecholic) structure possess antioxidant activity and there are a number of the phenolic constituents in olive oil which possess this structure, namely hydroxytyrosol and oleuropein which are the main phenolic constituents in olive oil. An *in vitro* evaluation of the antioxidant activities of olive oil and its separate constituents has been examined in numerous papers.

Saija et al. investigated the scavenging activities of hydroxytyrosol and oleuropein against the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and also a model compound, α-tocopherol, to help understand the effectiveness of antioxidants against the attack of oxygen radicals on biomembranes from the aqueous phase [6]. The model system consisted of dimyristoylphosphatidylcholine/linoleic acid unilamellar vesicles and a water-soluble azo compound as a free radical generator. The SC_{50} value of oleuropein was 25.22 μM and the corresponding value of hydroxytyrosol was 20.51 μM. It was hypothesized that this difference was because hydroxytyrosol can serve as scavenger of aqueous peroxyl radicals near the membrane surface, while oleuropein acts also as a scavenger of chain-propagating lipid peroxyl radicals within membranes.

There have been other studies which have investigated the scavenging effects of hydroxytyrosol and oleuropein with DPPH. These studies determined an EC_{50} value of hydroxytyrosol (which is equivalent to the SC_{50} value) of 26.0 μM and 19 μM [6,38]. The EC_{50} values of oleuropein in these studies were 36 μM and 22 μM respectively [6,38]. Gordon et al. also investigated the scavenging effects of oleuropein aglycone and hydroxytyrosol acetate [38]. Hydroxytyrosol acetate has been recently been reported to be found in olive oil [11]. The antioxidant activity of hydroxytyrosol acetate (26 μM) was higher than that of oleuropein and oleuropein aglycone (12 μM).

Briante et al. studied the antioxidant activity, in particular their capacity to inhibit the fatty acid peroxidation rate, of the main reaction products obtained after hydrolysis of oleuropein by hyperthermophilic β-glycosidase [39]. The main reaction products were oleuropein aglycone, hydroxytyrosol, elenolic acid with closed ring and elenolic acid with open ring. It was discovered that the antioxidant properties of the molecules tested are related to the degree of unsaturation of fatty acid. This study also confirmed that oleuropein and hydroxytyrosol possessed greater antioxidant capacity than elenolic acid in either form.

The radical scavenging potencies of homovanillic acid, homovanillic alcohol, hydroxytyrosol, the glucuronide conjugate of hydroxytyrosol and the sulfate conjugate of hydroxytyrosol with the radical DPPH have also been investigated. The SC_{50} values of homovanillic acid and homovanillic alcohol were found to be 14.8 and 11.4 μM. The glucuronide conjugate was a more potent than hydroxytyrosol with an SC_{50} of 2.3 μM, and the sulfate conjugate was almost devoid of radical scavenging activity [40].

The antioxidant activity of hydroxytyrosol and oleuropein aglycone has been investigated in humans. Human volunteers were administered phenol-rich olive oil which had increasing concentrations of catecholic compounds [44]. It was observed that the urinary excretion of hydroxytyrosol and its metabolite homovanillic alcohol were dose-dependent. The excretion of 8-iso-$PGF_{2\alpha}$ decreased with increased concentration of phenolics.

3.3. Antimicrobial properties of hydroxytyrosol, tyrosol and oleuropein

Bisignano et al. tested the in vitro susceptibility of oleuropein and hydroxytyrosol against several bacterial strains which were causal agents of intestinal or respiratory tract infections in humans [41]. Hydroxytyrosol and oleuropein both had antimicrobial activity against the ATCC bacterial strains. The minimum inhibitory concentration ($\mu g\ mL^{-1}$) of hydroxytyrosol and oleuropein, respectively, against theses bacterial strains is indicated in brackets after the bacterial strain. The strains tested were *hemophilus influenzae* ATCC 9006 (hydroxytyrosol 0.97, oleuropein 500), *moraxella ca-*

tarrhalis ATTC 8176 (hydroxytyrosol 1.92, oleuropein >500), *salmonella typhi* ATCC 6539 (hydroxytyrosol 3.94, oleuropein 125), *vibrio parahaemolyticus* ATCC 17802 (hydroxytyrosol 0.24, oleuropein 62.5) and *staphylococcus aureus* ATTC 25923 (hydroxytyrosol 7.85, oleuropein 62.5). Hydroxytyrosol and oleuropein were tested against clinical bacterial strains, they were cytotoxic to a large number of bacterial strains although to a lesser extent than the ATCC strains. The strains tested were *hemophilus influenzae* (hydroxytyrosol 0.96–15.60, oleuropein >500), *moraxwlla catarrhalis* (hydroxytyrosol 3.80–15.60, oleuropein >500), *salmonella* spp. (hydroxytyrosol 1.90–7.80, oleuropein 125–250), *vibrio parahaemolyticus* (hydroxytyrosol 0.97, oleuropein 125), *vibrio alginolyticus* (hydroxytyrosol 0.97–1.90, oleuropein 125), *vibrio cholerae* (hydroxytyrosol 1.90, oleuropein 125), *staphylococcus aureus* (hydroxytyrosol 3.9–31.25, oleuropein 62.5–125), *staphylococcus aureus* (hydroxytyrosol 3.9–31.25, oleuropein 31.25–125). It was postulated that although these biophenols have an *o*-diphenol system, which is responsible for the antibacterial activity of the olive phenols, the decrease in toxicity of oleuropein was due to its glycosidic group which may render it unable to penetrate the cell membrane or to reach the target site.

Oleuropein, hydroxytyrosol, caffeic acid and tyrosol were tested as scavengers of nitrogen species ($NO^{\cdot}$ and $ONOO^{-}$) nitric oxide. The observed scavenging of nitric oxide by hydroxytyrosol, oleuropein, and caffeic acid was concentration dependant over the range 5 μM to 75 μM. An inhibition of ~50% was achieved at a concentration of 75 μM. The compounds oleuropein, hydroxytyrosol and caffeic acid showed very substantial protection, in the range of 67 to 93% at 1 mM, against the effects of 0.5 mM $ONOO^{-}$. However, in both these cases tyrosol was less active than oleuropein, hydroxytyrosol and caffeic acid. This was explained by the lack of the catechol moiety in tyrosol [42].

Oleuropein has also been shown to have a protective effect on low density lipoprotein oxidazability in rabbits [19]. Rabbits were fed a diet enriched with olive oil and the amount of biophenols in plasma was then investigated. Rabbits were subjected to three different diets, the standard diet (diet A), addition of 10% (w/v) extra virgin olive oil (diet B) and diet C which contained the addition of 7 mg kg^{-1} of oleuropein to diet B. After ingesting the diet for 6 weeks, blood samples were obtained by intracardiac injection. The quantity of biophenol, vitamin E and C, uric acid and total, free and ester cholesterol were determined. Along with the amount of LDL. The inclusion of extra virgin olive oil (diet B) influenced the total biophenol content. The addition of oleuropein (diet C) increased this effect. The amount of vitamin E in rats fed diet C was minimally higher (4%) than diet B. However, the vitamin E content in the samples from diet B and C was nearly double to that of A (an increase of 76 and 83% respectively). Both diets B and C caused a reduction in the levels of vitamin C and uric acid in the plasma samples. It was observed that the administration of diet C lead to a 15% reduction in total cholesterol in comparison to diet B. This investigation verified the antioxidant capacity of oleuropein and other olive oil phenols but it also revealed that diet C increased the ability of LDL to resist oxidation and to reduce the plasmatic level of total, free and ester cholesterol.

In olive oil production a large amount of water is used that is named "waste water". This warm water is used to wash the olive paste prior to the separation of oil from this paste [43]. Olive mill waste water (OMWW) is currently disregarded due to the failure in developing a suitable "end-of-pipe treatment" technology to collect and recycle it [43]. In a recent study [43], three extracts from waste water were obtained and examined them for the presence of phenolic components. The extracts were obtained from OMWW after fractionation on an XAD 1180 resin (extract 1), after liquid-liquid extraction (extract 2) and liquid-liquid extraction followed by fractionation on a Sephadex LH-20 column (extract 3). The scavenging potencies of the extracts were examined with the free radical DPPH. Extract 1 had an EC_{50} value of 9.42 ppm, extract 2 was 3.12 and extract 3 was 1.83. The OMWW extracts were able to inhibit LDL oxidation and to scavenge superoxide anions and hypochlorous acid at concentrations as low as 20 ppm [43].

4. Absorption and metabolism of phenolics found in olive oil

4.1. In vivo studies on the constituents of olive oil

There have been several studies that have investigated the *in vivo* effect of the constituents of olive oil. The main ones of importance are discussed below. In situ intestinal perfusion on the absorption of oleuropein showed that oleuropein was absorbed under iso-osmotic conditions, with a permeability coefficient of 1.47×10^{-6} cm/s [46]. Under hypotonic conditions, the permeability of oleuropein was significantly greater (5.92×10^{-6} cm/s). 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 thus concluded that oleuropein could be poorly absorbed from the isolated perfused rat intestine.

Manna et al. investigated the kinetics of ^{14}C labeled hydroxytyrosol intestinal transport and metabolism in Caco-2 cells [28]. The uptake of hydroxytyrosol by Caco-2 cells was measured at 4°C and 37°C with increasing amounts of labeled hydroxytyrosol. It was observed that the rate of hydroxytyrosol was linear at both temperatures and consequently the intestinal transport system was not saturable and transport occurred by a passive diffusion mechanism. The P_{app} value calculated for Ap → BL transport was $12.4 \pm 0.9 \times 10^{-6}$ cm s^{-1} and the P_{app} value calculated for BL → Ap transport is $13.7 \pm 1.1 \times 10^{-6}$ cm s^{-1}. The similarity in these values indicates that the intestinal transport of hydroxytyrosol is bidirectional. It has been shown

that P_{app} values obtained *in vitro* on Caco-2 cells can be correlated with the absorption of drugs in humans, with 100% absorption occurring for P_{app} values greater that 10^{-6} [47]. From these results from Manna et al. it is likely that hydroxytyrosol will be 100% absorbed in humans. The only labeled metabolite found in this study was homovanillic alcohol (4-hydroxy-3-methoxy phenylethanol), a methylated derivative of hydroxytyrosol which is a product of intestinal COMT activity.

In 1998 the first paper which investigated the absorption of hydroxytyrosol in rats was published [23]. In this experiment 33 rats were divided into 11 groups. The rats were fasted overnight then orally administered of a solution of hydroxytyrosol (10 mg/ml in 0.5% tragacanth solution, 1 ml). At defined time intervals one group of rats was sacrificed and blood samples were collected. The hydroxytyrosol present in the plasma samples was pertrimethylsilylated and then analyzed by a validated GC-MS method. The results revealed that hydroxytyrosol appeared in plasma minutes after oral administration, with maximal concentrations of hydroxytyrosol obtained in the 5–10 min period and minimal concentrations of hydroxytyrosol observed after 60 mins. The concentration of hydroxytyrosol detected in the plasma was low in comparison to the amount administered however, no attempt was made in this experiment to look for the presence of metabolites. In this experiment the concentration of hydroxytyrosol fluctuated widely with the individual and one criticism of this paper is that there was no continuity with the study as rats were sacrificed in order for samples to be collected. This experiment was the first to imply that hydroxytyrosol could be absorbed into the bloodstream and consequently exert an antioxidant effect on the blood.

Visioli et al. has investigated the *in vivo* effect of hydroxytyrosol and tyrosol and in humans [30]. Phenol-poor olive oil was enriched with hydroxytyrosol and tyrosol and dosed to humans. The urinary levels of tyrosol and hydroxytyrosol were then determined by GC-MS after extraction and derivatisation of the samples. It was observed that the urinary levels of unconjugated tyrosol and hydroxytyrosol correlated with their intake, except at the highest dose which contained 1950 mg/L of total phenols, 84 μg/ml of hydroxytyrosol and 140 μg/ml of tyrosol. When the urine samples were treated with glucuronidase complete correlations between the amount of ingested and excreted hydroxytyrosol and tyrosol was observed. The proportions of hydroxytyrosol and tyrosol recovered in glucuronidase-hydrolyzed urine, with respect to ingested dose, were in the ranges of 30%–60% and 20%–22%, respectively. It was unclear as to the fate of the remaining amounts. This paper postulated that hydroxytyrosol and tyrosol were dose-dependently absorbed in humans and excreted in urine as glucuronide conjugates. It was also discovered that as the concentration of phenols administered increased the proportion of conjugation with glucuronide increased [30].

Upon re-examination of the urine samples two more metabolites of hydroxytyrosol, homovanillic acid (4-hydroxy-3-methoxy phenylacetic acid) and homovanillic alcohol, were identified by GC-MS (trimethylsilyl derivative) [45].

Visioli et al. investigated whether hydroxytyrosol prevented smoke-induced oxidative stress in rats [48]. It was known that during olive oil production large volumes of water are generated and discarded (see earlier). This waste water is known to contain large quantities of phenolic compounds and it was investigated if olive mill waste water would alter the urinary excretion of 8-iso-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) in rats exposed to passive smoking. 8-iso-$PGF_{2\alpha}$ was used as an indicator of oxidative stress-induced in vivo lipid peroxidation. In this study the olive mill waste water contained 8.3% of hydroxytyrosol and hydroxytyrosol was the only bioactive component present. The waste water was orally administered to rats *via* gavage and then exposed to cigarette smoke. It was discovered that exposure of rats to passive smoking increased the urinary excretion of 8-iso-$PGF_{2\alpha}$ by 44 $\pm$ 4.2% (n = 6) at 48 hr and by 55 $\pm$ 10%. Treatment of the rats with olive mill waste water extract prevented the increase at 48 hr and resulted in lower 8-iso-$PGF_{2\alpha}$ excretion at 96 hr. A later study showed that hydroxytyrosol, present in olive mill waste water, is dose-dependently absorbed and increases the antioxidant capacity of rat plasma [49] In this experiment three groups of rats were administered different amounts of olive mill waste extract. Their urine was collected for 24 hr, and the urinary levels of hydroxytyrosol were quantified by mass spectrometry. It was shown that hydroxytyrosol was dose dependently absorbed and was excreted in urine mainly as a glucuronide conjugate. This experiment also showed administration of olive mill waste extract increased the plasma antioxidant capacity.

The determination of hydroxytyrosol and tyrosol in human urine has been quantified by a capillary gas chromatography-mass spectrometry method [50,51]. In the earlier study the concentration of tyrosol in human urine were 2.0–47.4 μg/L in the pre-washout period and 2.4–25.2 μg/L during the washout period. The amount of tyrosol during the 24 hr after olive oil ingestion was 281–708 μg. Maximal tyrosol concentrations were observed in the 0–4 h urine samples. Tyrosol was excreted mainly in its conjugated form with only 6–11% excreted in urine as the free form. The recoveries of tyrosol in a 24 hr period were 24.7 $\pm$ 8.5%.

The later study reported the development and validation of an analytical method for the simultaneous determination of hydroxytyrosol and tyrosol in human urine after the intake of virgin olive oil. Prior to analysis by GC-MS the compounds were converted to their corresponding trimethylsilyl derivatives. Like the previous study maximal levels of tyrosol and also hydroxytyrosol were found in the first 4 hr after olive oil consumption. hydroxytyrosol recovered in its free form ranged from 20.5 to 76.2 μg (47.3 $\pm$ 17.5 μg; 5.9 $\pm$ 1.4%; mean $\pm$ s.d.). The amount of tyrosol recovered

in its free form ranged from 12.6 to 41.2 μg (28.6 μg ± 7.9; 13.8 ± 5.4%; mean ± s.d.). The amounts of hydroxytyrosol and tyrosol excreted in urine as free compounds never exceeded 15% and it was concluded that they were mainly excreted as conjugates. This paper also postulated that oleuropein is not the main source of hydroxytyrosol after ingestion of olive oil [51].

Tuck et al. investigated the *in vivo* fate of hydroxytyrosol and tyrosol after intravenous and oral dosing of either tritium labeled compound to rats [52]. Compounds were administered intravenously (in saline, tail vein) and orally (in oil- and water-based solutions), urine samples were collected and analyzed for total radioactivity. This study enabled the percentage of hydroxytyrosol or tyrosol and their conjugated metabolites eliminated in urine to be determined. For both hydroxytyrosol and tyrosol, the elimination of radioactivity in urine within 24 h for the intravenously and orally administered oil-based dosings was significantly greater than the oral, aqueous dosing method. There was no significant difference in the amount of phenolic compounds eliminated in urine between the intravenous dosing method and the oral oil-based dosing method for either tyrosol or hydroxytyrosol. This study also analyzed urine samples by HPLC-radiometric detection and it was observed that when hydroxytyrosol was dosed, hydroxytyrosol plus 5 metabolites were eliminated in urine. When tyrosol was dosed, tyrosol plus 1 metabolite were eliminated in urine. One major, unavoidable limitation of these studies was that it was done with rats and it is possible that hydroxytyrosol and tyrosol will be treated differently in humans.

Recently, the urine samples were re-examined and one metabolite of hydroxytyrosol has been identified as homovanillic alcohol, based on MS and NMR identification. Another two have tentatively been identified as the glucuronide and sulfate conjugate of hydroxytyrosol by MS analysis [40].

A recent article by D'Angelo et al. investigated the fate of radiolabelled hydroxytyrosol (^{14}C) in rats [53]. It was observed that there were no adverse effects at concentrations as high as 2 g/kg. Pharmacokinetic analysis revealed the hydroxytyrosol is absorbed quickly. Over 90% or the administered radioactivity is excreted in urine after 5 hr, which indicates that renal excretion represents the preferential disposition of hydroxytyrosol and/or its metabolites. About 5% of radioactivity was observed in faeces and gastrointestinal tract. In order to identify hydroxytyrosol metabolites the hydrosoluble labeled products were extracted from various organs and analyzed by reverse phase HPLC. The samples were also reacted with β-glucuronidase and sulfatase. From this it was deduced that hydroxytyrosol is converted to four oxidized and/or methylated derivatives. These metabolites were tentatively identified as homovanillic alcohol, homovanillic acid, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxyphenylacetaldehyde and sulfoconjugated derivatives. This study enabled the elucidation of basic pharmacokinetic parameters and allowed the metabolites of hydroxytyrosol to be identified.

It has therefore concluded that hydroxytyrosol is absorbed after oral ingestion and metabolized to numerous compounds at the present several compounds have been identified as the sulfate conjugate, glucuronide conjugate, homovanillic alcohol and homovanillic acid.

5. Conclusion

Although, there are numberous reports on the fate on the major phenolic constituents in olive oil, there are still numerous key issues that need to be answered and this will require further research. Namely, how exactly are these compounds absorbed and metabolized in humans. It has been postulated for decades that the consumption of olive oil has beneficial health effects. However, only recently the biological properties of its constituents have been investigated. Recent studies have confirmed these assumptions. Olive oil and its major phenolic components in olive oil, hydroxytyrosol, oleuropein and tyrosol are strong antioxidants and good radical scavengers. Oleuropein and hydroxytyrosol also show antimicrobial activity against ATTC bacterial strains and clinical bacterial strains. It has also been shown that the consumption of olive waste water prevented the increase in urinary excretion of 8-iso-$PGF_{2\alpha}$ to rats exposed to passive cigarette smoke. Several studies with hydroxytyrosol in Caco-2 cells and in rats have shown that hydroxytyrosol is metabolized to homovanillic acid, in rats it is metabolized to at least 5 metabolites. Two of the metabolites, homovanillic acid and homovanillic alcohol, are almost as strong radical scavengers as hydroxytyrosol. It is unclear at this time if the beneficial properties of olive oil are directly from its constituents or their metabolites. Further study in this area is required to determine the putatively beneficial properties of their metabolites.

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