Food Research International 105 (2018) 654–667

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Hydroxytyrosol: Bioavailability, toxicity, and clinical applications

María Robles-Almazan[a,1], Mario Pulido-Moran[b,d,1], Jorge Moreno-Fernandez[c,d,1], Cesar Ramirez-Tortosa[a], Carmen Rodriguez-Garcia[b,d], Jose L. Quiles[c,d], MCarmen Ramirez-Tortosa[b,d,*]

[a] Department of Pathological Anatomy, Hospital Complex of Jaén, Avenida del Ejército Español, 10, Jaén 23007, Spain
[b] Department of Biochemistry and Molecular Biology II, Faculty of Pharmacy, University of Granada, University campus of Cartuja, Granada 18071, Spain
[c] Department of Physiology, Faculty of Pharmacy, University of Granada, University campus of Cartuja, Granada 18071, Spain
[d] Institute of Nutrition and Food Technology José Mataix, University of Granada, Biomedical Research Centre, Avenida del Conocimiento, Armilla, Granada 18016, Spain

ARTICLE INFO

Keywords:
Hydroxytyrosol
Olive oil
Chronic diseases
Inflammation
Reactive oxygen species (ROS)
Cancer

ABSTRACT

Many beneficial properties have been attributed to the Mediterranean diet. Over the years, researchers have attempted to learn which foods and which food components are responsible for good health. One of these components is hydroxytyrosol, an important phenolic compound present in olive oil.

Hydroxytyrosol is a molecule of high interest to the pharmaceutical industry due to its anti-inflammatory and antimicrobial qualities its role against cardiovascular diseases and metabolic syndrome and for its neuroprotection, antitumour, and chemo modulation effects. The interest in this molecule has led to wide research on its biological activities, its beneficial effects in humans and how to synthetize new molecules from hydroxytyrosol.

This review describes the vast range of information about hydroxytyrosol, focusing on its involvement in biological mechanisms and modulation effects on different pathologies. This review also serves to highlight the role of hydroxytyrosol as a nutraceutical and as a potential therapeutic agent.

1. Introduction

The Mediterranean diet (MD) has been widely studied due to its association with improved human health. This diet is based on a high intake of cereals, vegetables, fruits, olive oil (OO), nuts, and legumes, a low quantity of meat and meat products, and a moderate amount of fish and seafood and moderate drinking of alcohol. It is well established that the MD is very effective against: cardiovascular disease (Estruch et al., 2006; Goncalves et al., 2015), diabetes (Salas-Salvado et al., 2014), inflammation (Martinez-Gonzalez et al., 2015), cancer (Couto et al., 2011), and aging (Gonzalez-Alonso et al., 2015; Varela-Lopez et al., 2015).

Many of the benefits associated with the MD are the result of a high intake of antioxidants and anti-inflammatory elements present in several components of this diet, especially with a high adherence to the diet (Diamanti et al., 2014; Gonzalez-Alonso et al., 2015; Granados-Principal et al., 2012).

There is much evidence of the health-beneficial effects of the MD because of the consumption of olive oil. This product contains large amounts of monounsaturated fatty acids and also antioxidants like (poly)phenols, which are responsible for the organoleptic characteristics, the auto-oxidation stability and all properties of olive oil in relation to health (Buckland & Gonzalez, 2015; Gonzalez-Alonso et al., 2015; Lopez-Miranda et al., 2010).

This review focuses on one of the most important components of olive oil's (poly)phenol group: hydroxytyrosol (HT).

Abbreviations: MD, Mediterranean diet; HT, hydroxytyrosol; IUPAC, International Union of Pure and Applied Chemistry; ADME, absorption, distribution, metabolism and excretion processes; EVOO, extra virgin olive oil; HTA, HT acetate; Tyr, tyrosol; EFSA, European Food Safety Authority; VECs, vascular endothelial cells; ROS, reactive oxygen species; Mn-SOD, Mn-Superoxide dismutase; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; CAT, catalase; cAMP, (cyclic adenosine monophosphate); cGMP, (cyclic guanosine monophosphate); PDE, phosphodiesterase; COX, cyclooxygenase; TxB2, thromboxane B2; TxA2, thromboxane A2; ICAM-1, Intercellular Adhesion Molecule 1; VCAM-1, vascular cell adhesion molecule 1; HAEC, aortic endothelial cells; TBARS, thiobarbituric acid reactive substances; LDH, lactate dehydrogenase; LDL, low density lipoproteins; NO, nitric oxide; TNF-α, tumour necrosis factor-alpha; PKC, protein kinase C; PGE2, prostaglandin E2; TGF-β1, transforming growth factor β1; Nrf-2, nuclear transcription factors NF-E2-related factor 2; HO-1, haem oxygenase-1; NQO-1, quinone oxidoreductase-1; BDNF, brain-derived neurotrophic factor; GAP43, growth associated protein 43; CDKs, cyclin-dependent kinases; FASN, fatty acid synthase; HER2, human epidermal growth factor receptor 2

* Corresponding author at: Department of Biochemistry and Molecular Biology II, Institute of Nutrition and Food Technology "José Mataix", Biomedical Research Centre, University of Granada, 18016 Armilla, Granada, Spain.
E-mail address: mramirez@ugr.es (M. Ramirez-Tortosa).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.foodres.2017.11.053
Received 4 July 2017; Received in revised form 12 November 2017; Accepted 19 November 2017
Available online 21 November 2017
0963-9969/ © 2017 Elsevier Ltd. All rights reserved.

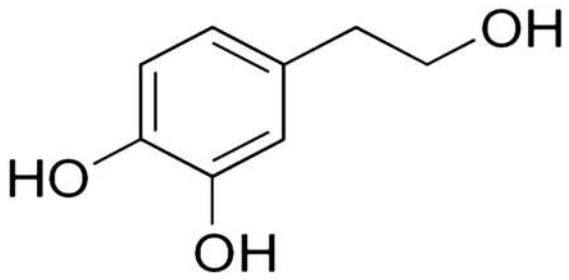


Fig. 1. Hydroxytyrosol structure.

2. Characteristics of HT

HT is an amphipathic phenol with a molecular weight of 154.16 g/mol and a phenylethyl-alcohol structure. It is also called 3,4-dihydroxyphenylethanol (DOPET) or 3,4-dihydroxyphenolethanol (3,4-DHPEA) or 4-(2-Hydroxyethyl)-1,2-benzenediol by the International Union of Pure and Applied Chemistry (IUPAC) system (Fig. 1). This compound is part of the soluble fraction from minority components of extra virgin olive oil and it is present at a very high concentration in the leaves of the olive tree (*Olea europaea L.*). HT is also one of the main compounds present in olive oil and it has been described as an antioxidant with many biological activities (Covas, de la Torre, & Fito, 2015; Perez-Bonilla, Salido, van Beek, & Altarejos, 2014). Due to the phenolic nature of HT, many studies have been carried out with different types of olive oils, enriched or not with this compound, to demonstrate its antioxidant, anti-inflammatory and antiatherogenic effects (Bernini, Merendino, Romani, & Velotti, 2013; Granados-Principal et al., 2014; Rodriguez-Morato et al., 2015). Additional studies have also focused on the antimicrobial (Medina, de Castro, Romero, & Brenes, 2006; Zoric et al., 2013) and dermatological properties (D'Angelo et al., 2005) of HT, thus showing the wide health-beneficial properties of this compound. Thanks to all these findings, HT has been postulated as a nutraceutical for preventing and treating different diseases.

The origin of HT is the hydrolysis of oleuropein which happens during the ripening of the olives, and during the storage and elaboration of table olives (Charoenprasert & Mitchell, 2012). When olives are processed to get their oil by crushing them, three different (poly) phenol-enriched layers are obtained: olive mill wastewater, pomace, and olive oil. Due to the amphipathic character of HT, it can be found on these three phases on a free form, as acetate form or as part of more complex compounds like oleacein, verbascoside, and oleuropein (Boskou, 2008).

On the other hand, the content of HT in olive oil depends on the kind of olive tree and olive, the location of the plantation, the oil quality, and the olive oil elaboration process (Romero et al., 2004).

HT is also present in the leaves of olive trees on a free form and also taking part in the structure of a complex of different elements. Some authors have determined that HT in VOO, and by-products of oil mechanical extraction process can be found mainly as aglycon derivatives of oleuropein and demethyoleuropein (3,4-DHPEA-EDA, 3,4-DHPEA-EA), doing very difficult to find HT as free form (Fabiani et al., 2006; Servili et al., 2004). Will be during the storage of the OO when the level of HT increase as a consequence of the hydrolysis of the secoiridoids. Other authors found that HT only was found in EVOO with a polyphenol content greater than 200 ppm (Montedoro, Servili, Baldioli, & Miniati, 1992). The extraction process of both HT and other (poly) phenols is commonly carried out in presence of methanol, ethanol or both, to induce the enzymatic activity of galactosidase to generate HT from oleuropein (Briante et al., 2002; Briante, La Cara, Febbraio, Patumi, & Nucci, 2002; Fernández-Mar, Mateos, García-Parrilla, Puertas, & Cantos-Villar, 2012).

In the last ten years, it has been found that HT is also present in red and white wines, with higher concentration in the first one. However, these concentrations are always lower than they usually are in extra virgin olive oil or extracts from leaves. The average concentration of HT found in the scientific literature for different type of wines and olive-leaf extracts (commonly employed as tisanes)(Di Tommaso, Calabrese, & Rotilio, 1998; Fernández-Mar et al., 2012; Minuti, Pellegrino, & Tesei, 2006; Quirantes-Pine et al., 2013; Ramirez-Tortosa, Pulido-Moran, Granados, Gaforio, & Quiles, 2014) are reported in Table 1. In spite of HT has been directly linked with OO, most of the authors have detected it in a very low concentration in VOO, reaching an average of 1.8 mg of HT per kg of 210 different VOO evaluated samples, obtained in industrial plants (Servili et al., 2004). Similar results to Servili et al. (2004) were obtained by Ocakoglu, Tokatli, Ozen, and Korel (2009). Other authors showed that the concentration of free HT on a Picual variety of OO could only reach values around 200 µol per kg of OO after seven months of storage (García, Brenes, Romero, García, & Garrido, 2002).In addition, Godoy-Caballero, Acedo-Valenzuela, and Galeano-Diaz (2012) showed a range of concentration of HT from 9 up to 21 µg of HT per gram of OO, depending on the variety of them.

Table 1
Average concentration of HT in different foods.

Food	HT average content
White wine	2 mg/l
Aged red wine	20 mg/l
Red wine	3 mg/l
Olive tree leaves	12 mg/g

In contrast, other authors showed a high that the concentration of HT can oscillate from 0.5 to 60 mg of HT per kg of OO (Alarcón Flores, Romero-González, Garrido Frenich, & Martínez Vidal, 2012). Similar results were obtained by García-Villalba et al. (2010), where HT achieved up to 20 mg per kg of OO. This large discrepancy may be due in part to differences in extraction procedures and chromatographic methods employed for the analysis of the phenolic compound from VOO (Servili et al., 2004). To date, this issue and the development of methodologies to quantify phenols in EVOO have been extensively discussed (García-Villalba et al., 2010).

Nowadays, many researchers are looking for new forms of HT to improve the ADME (absorption, distribution, metabolism and excretion) processes, its stability and its health-biological features. These studies are focused on changing the solubility of HT in order to increase its bioavailability and its plasma half-life (Mateos et al., 2011). Almost all experiments have been carried out in *in vitro* models. Some of these new isolated compounds, their biological activities and their synthesis methods have been reviewed by Bernini et al. (Bernini et al., 2015):

HT Esters: the most important compound of this group is HT acetate which is found in extra virgin olive oil. HT acetate has demonstrated a higher antioxidant capacity than HT. In addition, nitro-ester derivatives have shown beneficial effects in Parkinson's disease associated to their antioxidant properties (Trujillo et al., 2014).

HT Alkyl Ethers: a new class of lipophilic HT derived with high cytotoxic activities in A549 lung cancer cells and MRC5 non-malignant lung fibroblasts (Calderon-Montano et al., 2013).

HT analogues: with similar structure to HT but different substituents on the aromatic ring and/or a different length of the alcoholic chain.

HT thioderivatives: containing thioacetate, thiol, and disulphide groups.

HT derived isochromans: also presents in extra virgin olive oil methanolic extracts.

3. How are absorption, distribution, metabolism and excretion (ADME) processes?

It is well stablished that phenolic compounds are absorbed in a dose-dependent manner in the bowel and they suffer an important intestinal/hepatic metabolism (Granados-Principal, Quiles, Ramirez-Tortosa, Sanchez-Rovira, & Ramirez-Tortosa, 2010; Rubio et al., 2012; Visioli et al., 2003). Many studies have been performed in animals and humans in order to determine how the ADME processes of HT occur. These studies have shown similar ADME characteristics for HT with other (poly)phenols. In this sense, HT presents different ways of absorptions and excretion depending on the vehicle employed (Gonzalez-Santiago, Fonolla, & Lopez-Huertas, 2010; Tuck, Freeman, Hayball, Stretch, & Stupans, 2001).

All (poly)phenols suffer the phase I of metabolism (hydrolysis) in enterocytes and subsequently go through phase II of metabolism, where they are transformed into glucuronide, methylated and sulphate by-products (Suarez et al., 2011). For all processes described above, the 98% of HT can be detected in plasma and urine in glucuronide form, and only 2% as its free form when it is administered in olive oil form (Miro-Casas et al., 2003).

More recent studies were conducted to determine different specific (poly)phenolic metabolites that could explain more concisely the biological activities associated to some components of the MD as well as to establish some better compliance markers. In this sense, Rubió et al. (2014) observed in hypercholesteraemic volunteers that after the administration of two different phenolic-enriched EVOO, the most quantifiable and appropriate biomarker for compliance were the sulphate forms of HT, measured both in urine and plasma samples.

Finally, a more complex study carried out by Khymenets et al. (2016) allowed to corroborate that after the administration of a HT nutraceutical preparation to healthy volunteers, sulphate metabolites of HT and concretely the HT-sulphate-3′ could be selected as the best biomarker of compliance of olive oil ingestion.

3.1. Absorption process

The absorption of HT mainly occurs by passive transport in the small bowel and the colon with an efficiency that oscillate from 75% up to 100%. The absorption process depends on the vehicle employed, being more effective in the form of olive oil (Tuck et al., 2001). These results agrees with Vissers et al. studies (Vissers, Zock, Roodenburg, Leenen, & Katan, 2002), which reported an absorption of 70% of HT in patients in whom an ileostomy had been carried out. It is well known that phenolic bioavailability is influenced by different factors such as age, hormonal status (Roowi, Mullen, Edwards, & Crozier, 2009), or gender, as described by de Bock et al. in 2013 when leaf extracts were administered to determine the perimenopause activity of HT (de Bock et al., 2013). In addition, Dominguez-Perles, Aunon, Ferreres, & Gil-Izquierdo in 2017, described that gender is a critical feature for the final bioavailability of HT derivatives, persisting this compound for a longer time in the body of female rats after different forms of oral administration of HT.

Furthermore, other studies have demonstrated that HT achieves its highest urinary levels when it is administered as red wine, probably due to the interaction between ethanol and dopaminergic ways. HT is a dopamine metabolite (Fig. 2). The increased levels of HT in urine, when it is administered as red wine, can be consequence of a higher boost to dopamine metabolism (de la Torre, Covas, Pujadas, Fito, & Farre, 2006). In the light of the results of Perez-Mana et al. (2015) studies, which demonstrated that if HT is administered with ethanol, dopamine metabolism varies to produce HT instead of DOPAC (3,4-dihydroxyphenylacetic acid). The increasing levels of HT in the body can be easily comprehended.

3.2. Distribution of HT

This compound has a swift absorption process and reaches its maximum plasma concentration around 7 min after the intake (Bai, Yan, Takenaka, Sekiya, & Nagata, 1998). In spite of this, other authors have suggested that the highest plasma concentrations of HT are reached between 0.5 and 1.0 h after its oral administration being practically undetectable after 4 h. In this sense, when HT is administered in an acetate form, the highest plasma concentration of HT is reached between 0.5 and 2 h after oral administration (Dominguez-Perles, Aunon, Ferreres, & Gil-Izquierdo, 2017). Once absorbed, HT will quickly start being part of plasmatic high density lipoproteins, acting as an antioxidant and as a cardiovascular protector (Fernandez-Avila et al., 2015).

Because of the powerful and quick metabolism of HT, this compound presents a plasma half-life of 1–2 min (D'Angelo et al., 2001; Granados-Principal et al., 2014). HT and its metabolites have very good distribution abilities in tissues such as muscle, testis, liver, and brain (HT is able to cross the blood brain barrier) besides it is accumulated in kidney and liver (D'Angelo et al., 2001). This widespread distribution is responsible for the health-beneficial properties of HT (Serra et al., 2012).

A current study has demonstrated that free hydroxytyrosol in human plasma is practically undetectable when it is originated from ordinary olive oil. Conversely, high plasma levels of free HT can be detected after the administration of extra virgin olive oil (EVOO) (Pastor et al., 2016).

The fact that HT is also found in brain tissue (thanks to its ability to cross the blood brain barrier) and its already described possible interaction with dopaminergic pathways, suggests a role as a dopaminergic neuronal protector (Schaffer, Muller, & Eckert, 2010) due to its ability to increase the antioxidant defences and for its free radicals scavenger capacity (Hashimoto et al., 2004). both effects demonstrated in *in vivo* and *ex vivo* models.

In summary, differentiating the exogenous and endogenous sources of HT in the body becomes a difficult task consequence of the wide distribution of HT, its short plasma half-life and its possible interaction with different ways such as dopamine pathway (de la Torre et al., 2006; Mosele et al., 2014).

3.3. Metabolism of HT

HT has an intense and rapid metabolism. Firstly, this compound suffers the first step of its metabolism inside enterocytes and subsequently in the liver. In this sense, gut microbiota has been identified as a notably modulator of absorption both of HT and its metabolites. This microflora acts transforming part of HT, HT acetate (HTA), and tyrosol (Tyr) in different catabolites HT-derived, and releasing free-HT from its more complex forms. According to Mosele et al. (2014), the metabolism of HT by microbiota occurs firstly by oxidation and this is followed by a transformation into hydroxylated phenylacetic acids.

In plasma, free HT can be found in a very low concentration, ortho-methyl products of HT (homovanillic alcohol and acid). glucuronide derivatives, glutathionyl conjugates and sulphate derivatives (Rodríguez-Morató et al., 2016). Moreover, the study carried out by Kotronoulas et al. in 2013 determined, in rat urine samples, a new metabolite called *N*-acetyl-5-S-cysteinyl-hydroxytyrosol which derived from HT mercapturate, resulting from the HT oxidation pathways (Kotronoulas et al., 2013).

D'Angelo et al. in 2001 established that HT was enzymatically converted into oxidized and methylated derivatives: 3,4-dihydroxyphenylacetaldehyde, 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid and 4-hydroxy-3-methoxyphenylethanol when HT was administered in a single intravenous dose of 1.5 mg/kg–87.5 μCi/kg. All these transformations can be resumed in two abbreviated forms as Fig. 3 shows:

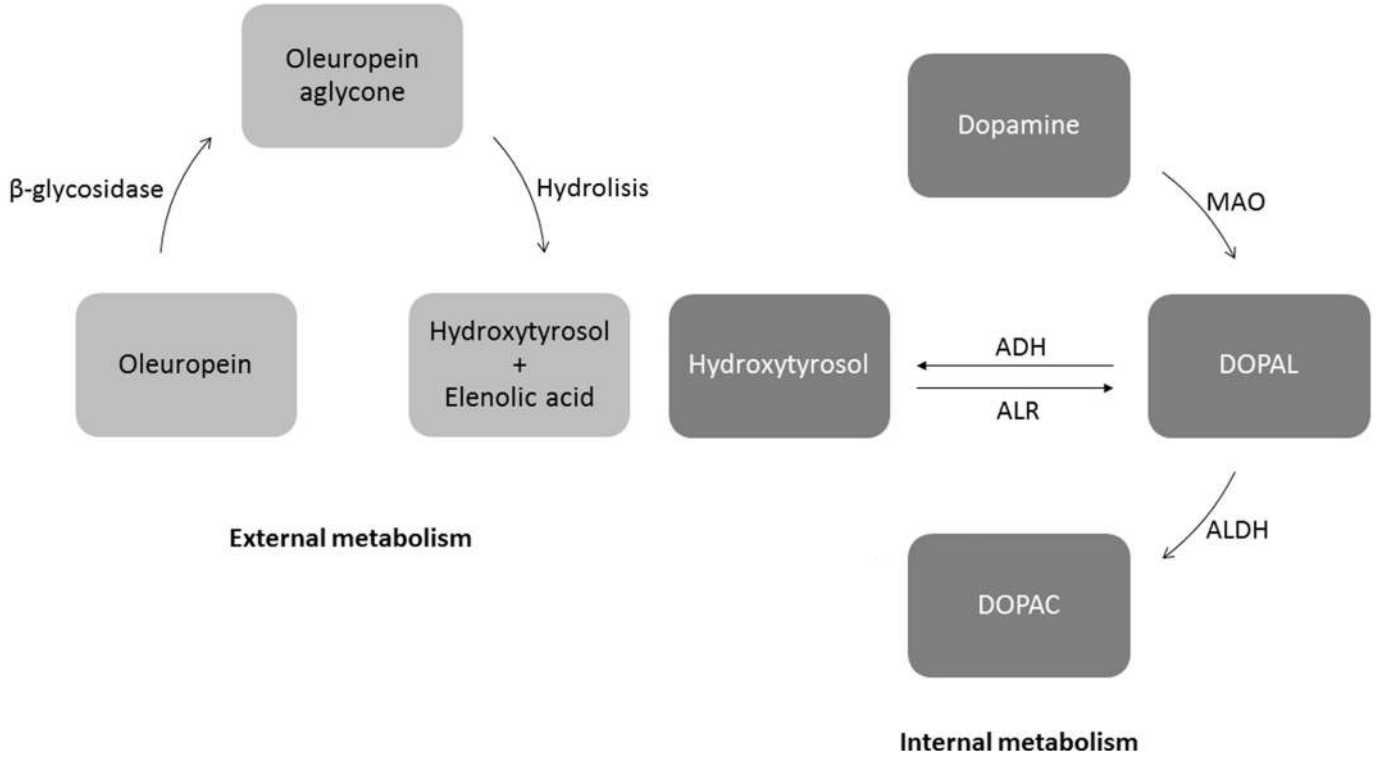


Fig. 2. Comparison between internal and external metabolism of hydroxytyrosol. Abbreviation: ALR: aldehyde reductase; ADH: alcohol dehydrogenase; MAO: monoamine oxidase; ALDH: aldehyde dehydrogenase; DOPAL: 34-dihydroxyphenylacetaldehyde; DOPAC: 34-dihydroxyphenylacetic acid.

Oxidation: alcohol and aldehyde dehydrogenases produce 3,4-dihydroxyphenylacetaldehyde and subsequently 3,4-dihydroxyphenylacetic acid.

Methylation: catechol ortho methyl transferase produces 4-hydroxy-3-methoxyphenylethanol also called homovanillic acid.

Finally, all these compounds are transformed into sulfo conjugates by a sulfotransferase enzyme.

On the other hand, recent studies performed suggest that HT can derive to tyrosol instead of homovanillic acid as a consequence of the transformations promoted by gut microflora (Dominguez-Perles et al., 2017). In the same study, it was also found that the metabolism of HT depends on the gender, being more efficient the transformation and utilization of HT by females (Serra et al., 2012).

3.4. Excretion

Taking into account the structure of HT and its intense metabolism and transformations, conjugated catabolites are mainly excreted by the kidneys (Visioli et al., 2003). The time required for the complete elimination from the body, both for HT and its metabolites is approximately of 6 h in humans (Rodríguez-Morató et al., 2016; Suarez et al., 2011) and around 4 h in rats (Dominguez-Perles et al., 2017). In kidney, HT is accumulated until its excretion (D'Angelo et al., 2001), where it may perform a nephroprotective role thanks to its antioxidant properties (Chashmi, Emadi, & Khastar, 2017). D'Angelo et al. in 2001 established that around 5% of total HT is excreted by faeces after 5 h of an injection of HT (1.5 mg/kg–87.5 μCi/kg).

4. Toxicity and dose establishment

In spite of HT being part of natural and healthy food such as olive oil, there are several studies which were carried out to demonstrate the toxicity of HT. This toxicity has been measured in cells and animal models by assessing the acute toxicity, teratogenicity, mortality, morbidity or mutagenic effects (Aunon-Calles, Canut, & Visioli, 2013; Christian et al., 2004; Lee et al., 2016; Soni, Burdock, Christian, Bitler, & Crea, 2006). Nowadays, there is a great interest in using HT as a possible nutraceutical compound against different diseases such as cancer, diabetes or chronical pathologies, although further studies must be performed to understand the possible toxicological effects of HT in human. In this sense, we highlight the scarce literature available in this respect in humans. As consequence of its poor bioavailability, there are no studies that determine if the final blood concentration could have toxic effects after the intake of a high concentration of HT, both as pure extract and as part of enriched foods such as EVOO. In this sense, some authors could determine null toxicity in the first gastrointestinal tract with other (poly)phenols (Babich & Visioli, 2003). To date, no toxicity studies have been carried out to stablish if HT could exert any toxicological effect at this level, but if we take into account the descriptions of the toxicity studies carried out and also described above, and the high similarity between the behaviour of HT and other phenolic compounds, we have a fairly accurate approximation of its null toxicity at all levels, although more studies have what to do to conclude this claim in humans.

In relation to the study of acute and subchronic toxicity, HT has been studied commonly as olive oil, olive mill wastewater forms, and as extracts in animals and cells. In this sense, there are numerous studies performed in rats with different administrations ways to determine the acute toxicity. Some authors have described that the injection (intravenous, subcutaneous or intramuscular) of 2 g/kg in rats provoked piloerection and local redness in the inoculation area disappearing this effects before 48 h (D'Angelo et al., 2001).

A very interesting study with HIDROX™ (Hydrolysed Aqueous Olive Pulp Extract; OPE) an aqueous olive-pulp extract with a high content of HT (around 70%) administered by oral gavage in Sprague Dawley rats and CD1 ICR (BR) mice was performed by Christian et al. in 2004. The dose of HT employed was risen to 2 g/kg (equivalent to around 1400 mg/kg of pure HT) to determine the acute toxicity. These authors observed a null mortality and morbidity. The only alterations proven was the presence of diarrheal faeces in rats. This study proposed a LD50

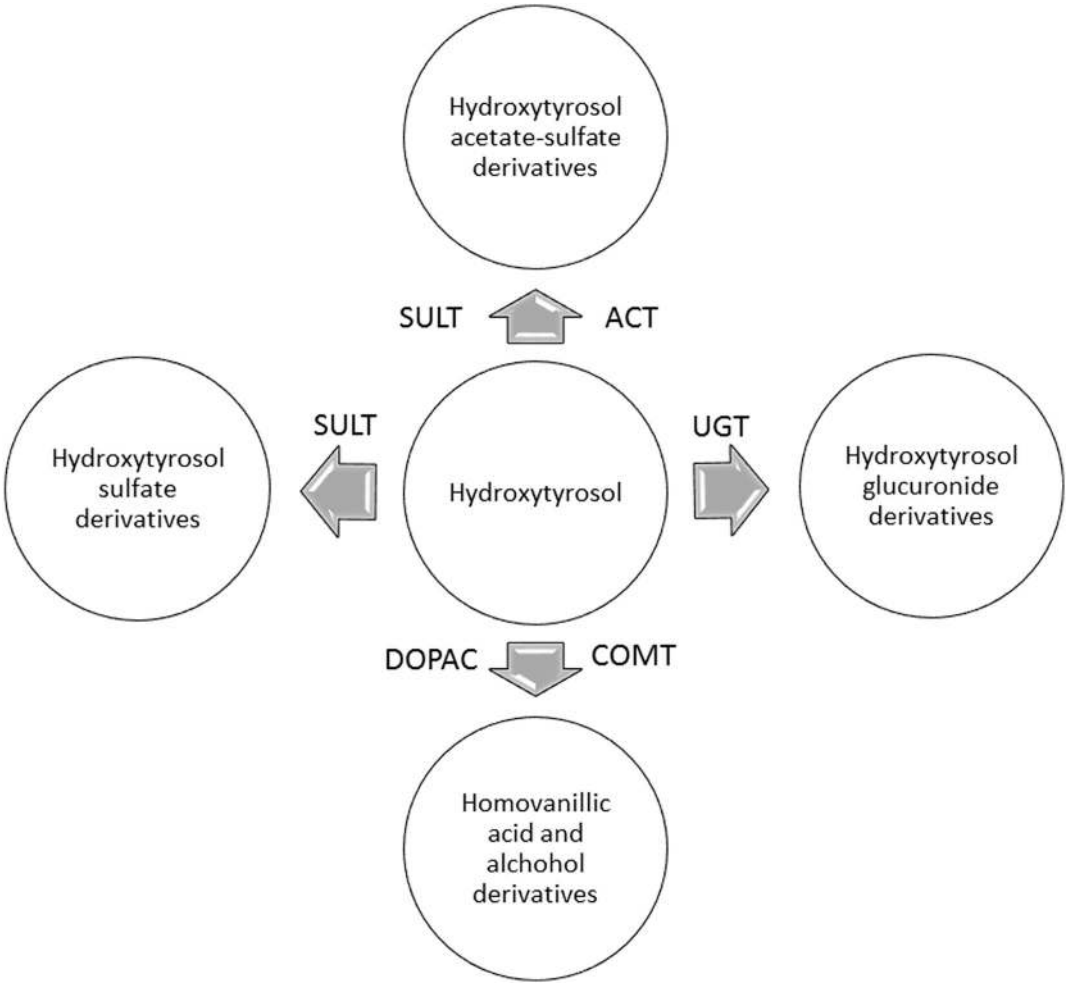


Fig. 3. Biotransformation pathways of hydroxytyrosol. Abbreviations: COMT: catechol-O-methyltransferase; UGT: UDP-glucuronosyltransferase; SULT: sulfotransferase; ACT: O-acetyltransferase.

of 2000 mg/kg for HYDROX™, representing a median lethal dose (LD50) of around 1400 mg/kg for pure HT. Besides these results, Christian et al. in 2004 employed a higher dose in rats (raising 5 g/kg) and they established a LD50 of around 3.5 g/kg of HT.

Furthermore, to evaluate subchronic toxicity, other parameters such as body weight, food intake, haematology and histopathology from selected tissues, physical and ophthalmic characteristics were also analysed. No remarkable changes associated to HT or to the rest of HIDROX's compounds were observed at the end or during the study.

Aunon-Calles et al. (2013) performed a study to evaluate the toxicological effects of pure hydroxytyrosol. This study reported that the oral administration of HT once a day for 13 weeks at doses of 5, 50, and 500 mg/kg/d did not lead to any micro- and macroscopic alterations or to death. They observed a gain in body-weigh without interest for toxicological analysis, and higher kidney weighs in the group with the highest treatment dose, with no changes in the organ functionality or structure. Therefore, they came to the conclusion that no adverse effects were observed at doses of 500 mg/kg/d. Additionally, Aunon-Calles, Giordano, Bohnenberger, and Visioli (2013) did not observe any sign of genotoxic and mutagenic effects when HT was employed in *in vitro* models at physiological concentration.

One of the last studies performed in animal models to determine the subchronic toxicity of HT was carried out by Heilman et al. (2015). These authors used an extract called H35 (35% w/w HT). This extract was administered to female and male Wistar rats by oral gavage for 90 consecutive days, at doses of 125, 250 and 500 mg HT/kg/day. There were no toxicologically significant effects found. These researchers did not observe any adverse effects for 250 mg HT/kg/day. Similar study conditions were used by Kirkland, Edwards, Woehrle, and Beilstein (2015). These authors used a H40 extract (with a 40% of HT total content) and no mortality rate was observed, although a decrease in body weight and an increase in relative weight of liver, thymus, kidneys and spleen were found in male rats.

On the other hand, HT presents a pro-apoptotic and antiproliferative effects in different types of tumour cells when these cells are treated with pure HT or extract forms. As a result of the pharmacomodulation that have been performed with HT, its selectivity and efficiency on tumour cells has been enhanced by preventing or diminishing their proapoptotic activity on human non-tumour cells (Burattini et al., 2013; Calderon-Montano et al., 2013). In addition, Anter et al. (2014) demonstrated how a simple extraction from olive mill wastewater also called “alperujo” or using it directly, can exhibit a notable antiproliferative and caspase 3-dependent proapoptotic effects against the human tumour cell line HL60, avoiding its cell toxicity in non-tumour cells.

Take in a count all data described above and to the health-beneficial effects in human cardiovascular diseases, the European Food Safety Authority (EFSA) since 2011, approved health claim on olive oil polyphenols and recommended the daily consumption of 5 mg HT and its derivatives (*e.g.* oleuropein complex and tyrosol) provided by moderate amounts of EVOO (20 g) to maintain a balanced diet. This dose is enough to generate health-beneficial properties by decreasing low density lipoproteins oxidation, increasing high density lipoproteins, maintaining a normal blood pressure, and avoiding the pro-inflammatory

processes. In spite of the daily dose of HT seems to be low, we have to note that, according to the scientific literature described above, the average concentration of HT in VOO could be so far from the prescription stablished by the EFSA, and a high intake of OO would be necessary to reach the recommended beneficial levels of HT.

5. Main activities associated to HT

5.1. Antimicrobial effects of HT

Extracts from olive oil and olive tree leaves have been used as soap, solutions or balms for a long time to palliate several pathologies associated with infections caused by microorganisms (Medina et al., 2006).

It is well known that both olive tree leave's extracts and pure HT have a powerful antimicrobial activity against some microorganisms such *Escherichia coli*, *Candida albicans*, *Clostridium perfringens*, *Streptococcus mutans*, or *Salmonella enterica* (Medina et al., 2006; Zoric et al., 2013).

Not only that, some studies have demonstrated in *in vitro* models a helpful effect of HT against *Vibrio cholerae*, *Salmonella typhi*, *Haemophilus influenza* or *Staphylococcus aureus* even at lower dosages than some antibiotics such as ampicillin (Bisignano et al., 1999). It has also been described an antiparasitic activity by HT against several parasites such as *Lesishmania spp*. (Kyriazis, Aligiannis, Polychronopoulos, Skaltsounis, & Dotsika, 2013).

A recent study has determined that HT has an effective antimicrobial effect against *Staphylococcus aureus* and a relevant role on prevention and treatment of biofilm-based infections, and also with infections related to medical devices or chronic wounds, thanks to the ability of HT to decrease the production of reactive oxygen species (ROS) by microbial biofilms, as well as for its ability to avoid microbes adhesion (Crisante et al., 2015), showing its possible role to avoid nosocomial diseases in hospitals and other care institutions. Despite of all these studies, other authors has suggested a microbiocidal limited capacity of the HT, showing some controversy regarding its antimicrobial effect (Medina-Martinez, Truchado, Castro-Ibanez, & Allende, 2016) so that further studies are needed to clarify if its use as an anti-infective in humans is possible in a few years.

5.2. HT as ROS scavenger

One of the most important activities associated to HT is its role as ROS scavenger. HT and its metabolites have a high capacity to eradicate both intracellular and extracellular production of ROS. At an extracellular level, HT and derivatives have shown a ROS scavenger activity in skin cells by blockading the harmful effects of ROS. This capacity is important when ROS are generated by UV radiation (Zwane, Parker, Kudanga, Davids, & Burton, 2012) or by peroxynitrite radical (Deiana et al., 1999), both able to break DNA strands and to promote genotoxicity. Intracellularly, HT has also an extraordinary ROS scavenger capacity, particularly with free radical molecules such as superoxide, hydrogen peroxide, and hypochlorous acid, as well as acting as a metal chelator (Granados-Principal et al., 2012; Granados-Principal et al., 2014; Manna et al., 1999).

Some studies have proposed different mechanisms of action for HT related to antioxidant pathways such as ARE, which finally leads to the modulation of nuclear factor-E2-related factor-2 (Nrf2) and JNK-p62/SQSTM1 (Bayram et al., 2012; Zou et al., 2012). HT also acts as mitochondrial biogenesis stimulant by increasing PPARGC1α (Zhu et al., 2010). A similar pattern has been observed in vascular endothelial cells (VECs) where HT protects the cells against the cytotoxic effect of hydrogen peroxide by activating Akt and ERK1/2. In addition to this, HT increases the expression and nuclear translocation of Nrf2 (Zrelli et al., 2011), then promoting the activation of multiple genes encoding antioxidant response element(s) (ARE) such us DNA-repair proteins or phase II detoxifying enzymes. Other authors, however, did not find the evidence that HT could activate Nrf2 when the study was carried out in humans, showing the need to carry out further clinical trials to clarify it (Crespo et al., 2015; Visioli, 2015).

Studies carried out in normal human fibroblasts have demonstrated the beneficial effects of HT in mitochondria, caused by the increase of Mn-Superoxide dismutase (Mn-SOD) activity and by the decrease age-associated of mitochondrial ROS accumulation (Buettner, Ng, Wang, Rodgers, & Schafer, 2006; Sarsour et al., 2012). These studies confirmed the mitochondrial protective role of HT (Fabrizio, Pletcher, Minois, Vaupel, & Longo, 2004; Sarsour et al., 2012). In addition, HT influences on sperm motility and viability. The application of HT to spermatozoa cultures improves sperm viability and decreased both sperm DNA oxidation and ROS levels (Kedechi et al., 2016), but still unclear if the employment of HT *in vivo* could have any beneficial effect in terms of sperm viability or fertilization techniques.

On the other hand, HT can produce a direct action over the antioxidant system by increasing SOD, glutathione peroxidase (GPx) or glutathione reductase (GR) activities and also maintaining higher levels of reduced glutathione (GSH) in cells (Granados-Principal et al., 2014; Li et al., 2012), fact that could be associated with a possible parahormetic effect of HT, as described below. Besides, HT heightens catalase (CAT) activity, favouring the removal of peroxides from cells environment (Hashimoto et al., 2004). In addition, several authors have associated this antioxidant capacity of HT with its already described neuroprotective effects (Koo, Kim, Oh, & Kim, 2006; Rodriguez-Morato et al., 2015; Zheng et al., 2015).

Although many of the studies described above showed an antioxidant beneficial effect of HT, other authors concluded that the administration of HT in its OO form in healthy humans did not exert any significant antioxidant effects associated to its glucuronide metabolites (Khymenets et al., 2010), thus proposing that the concentration of HT present in OO could be insufficient to promote an antioxidant effect. This fact demonstrate the imperious need to conduct more *in vivo* studies that allow to determine what is the optimal intake of HT to reach a physiological concentration that promote health-beneficial effects of HT in humans.

Even though all mentioned studies have been focusing on the role of HT as ROS scavenger, this compound can also increase oxidative stress levels in colon cancer cells (DLD1 cells) but not in normal colon epithelial cells (CRL1807 cells). This may help to understand the two main activities of HT: antioxidant and selective antitumour capacity. So there is a certain dichotomy in how HT affects cells, based on how HT runs its action (Atzeri et al., 2016; Maalej, Bouallagui, Hadrich, Isoda, & Sayadi, 2017; Nogueira et al., 2008). In the study performed by Sun, Luo, and Liu (2014), they reported that HT treatment increased Akt activity, which later sensitizes cells to get a selective killed by oxidative stress-induced apoptosis. Another similar study performed in cells by Luo et al. (2013) demonstrated an identical mechanism of action of HT during ROS formation, only against the human prostate cancer cells (PC-3, DU145) and not in an immortalized non-malignant prostate epithelial cell line (RWPE-1). Moreover, an extra input of extracellular superoxide and hydrogen peroxide facilitated the anti-proliferation effect of HT in prostate cancer cells.

Similar results were obtained by Rosignoli, Fuccelli, Sepporta, & Fabiani in 2016 who observed, in different types of cancer cell lines: MDA and MCF-7 (breast). LNCap and PC3 (prostate), SW480 and HCT116 (colon), how HT performed its antitumour activity by accumulating hydrogen peroxide in the culture medium, acting in this case as a prooxidant-antitumour compound in cancer cells.

To date, there is a certain trend, increasingly questioned, that the antioxidants supplements directly generate an antioxidant effect and are therefore beneficial for the body. This fact has been discussed for years and there seems to exist some contradiction in this respect (Bjelakovic, Nikolova, & Gluud, 2014). The current trend focuses on investigating the possible effects of para-hormesis of dietary antioxidants and how they use the antioxidant enzymatic system to exert

their health-beneficial effects in the organism, thus acting in an indirect way, in contradiction with the postulate to the present (Forman, Davies, & Ursini, 2014).

Multiple studies have been discussed in this review demonstrating the antioxidant functions of HT. Nevertheless, the doses used have not been standardized and there are not many studies in humans to clearly state the HT antioxidant pattern. In this sense, the only study performed in humans with pure HT to date did not show any remarkable effects of this compound at high doses (Lopez-Huertas & Fonolla, 2017). Therefore, we must be cautious to affirm categorically that the supplementation of antioxidants and particularly of HT propitiates *per se* an antioxidant beneficial effect in the organism. In addition, we also describe in this work a certain dichotomy between the antioxidant and pro-oxidant effect of HT when used as an antitumor compound.

5.3. Role of HT in cardiovascular system

Thanks to its already described antioxidant effect, HT avoids the oxidation of low density lipoprotein (LDL). In consequence, it decreases the formation of atherosclerotic plaques (Gonzalez-Santiago et al., 2006; Granados-Principal et al., 2012; Warleta et al., 2011). Some HT-enriched extracts have also reduced plasmatic levels of total cholesterol and lipids (Jemai, Bouaziz, Fki, El Feki, & Sayadi, 2008; Poudyal, Campbell, & Brown, 2010) and blood pressure (Khayyal et al., 2002) decreasing cardiovascular risk factors. This is the main reason why the EFSA recommends a daily ingestion of at least 5 mg of HT (usually in olive oil form).

In addition to all, HT can be considered as an antithrombotic and anti-inflammatory molecule (Cicerale, Conlan, Sinclair, & Keast, 2009) due to its ability to impede the platelet synthesis through cAMP- (cyclic adenosine monophosphate) and cGMP- (cyclic guanosine monophosphate) phosphodiesterase (PDE) inhibition (Medina-Martinez et al., 2016). Besides, HT can inhibit the cyclooxygenase (COX) (Richard et al., 2011; Zhang, Cao, & Zhong, 2009) decreasing thromboxane B2 (TxB2) and thromboxane A2 (TxA2) production (de Roos et al., 2011; Leger et al., 2005).

HT and its metabolites have also the ability to decrease the secretion of different adhesion molecules such as *E*-selectin, P-selectin, Intercellular Adhesion Molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) in human aortic endothelial cells (HAEC) (Catalan et al., 2015) being both HT and its synthetized metabolites responsible for protection against endothelial dysfunction, thus decreasing, at least in part, the risk in the early stages of atherosclerosis. A recent study showed that HT and secoiridoids regulate the expression of several proteins both in aorta and heart tissues, which are involved in the proliferation and migration of endothelial cells, the occlusion of blood vessels, and cardiac functions, having both components of EVOO a pivotal protective role in the cardiovascular system (Catalan et al., 2016).

Several cardiovascular disorders are consequence of drugs administration such as doxorubicin in cancer treatment. In this sense, Granados-Principal et al. in 2014 showed for the first time in a rat *in vivo* model that HT could improve the cardiac disorders generated by a ROS imbalance production and mitochondria damage caused by doxorubicin-based therapy. Therefore, HT could be used as an adjuvant molecule in antitumor therapies that cause an increase in cardiovascular risk by themselves, also facilitating an improvement in the antitumor response and a lower number of adverse effects associated with the treatment.

Some nutrigenomics studies have been performed to determine what genes involved in cardiovascular disorders such as atherosclerosis or insulin sensitivity mechanisms could be altered after the administration of OO and HT. In this sense, Khymenets et al. (2009) observed in peripheral blood mononuclear cells of healthy volunteers after the administration of 25 ml of VOO for three weeks, the upregulation of ten genes (ADAM17, ALDH1A1, BIRC1, ERCC5, LIAS, OGT, PPARBP, TNFSF10, USP48, and XRCC5) related to the development and progression atherosclerosis processes. A further study also performed in peripheral blood mononuclear cells of healthy volunteers, revelled the modulation of seven genes related to insulin sensitivity mechanisms (ADAM17, ADRB2, ALOX5AP, CD36, LIAS, OGT and PPARBP), after the administration of 50 ml of VOO (Konstantinidou et al., 2009).

Although these studies did not directly relate the administration of HT to the results obtained, it must be noted that HT is one of the main components of olive oil causing many of these health-beneficial effects. In addition, it should be taken into account that the modulation of gene expression does not imply a direct variation in the biochemical parameters, but is conditioned by multiple adjacent factors. Therefore, a greater number of studies should be carried out to determine if these gene modifications are reproducible and associable to the HT *per se*, as well as to deepen the knowledge of how this modulation is produced.

5.4. How acts HT in metabolic syndrome?

Metabolic syndrome is described as a combination of different abnormalities that includes obesity, dyslipidaemia, impaired glucose tolerance, insulin resistance, diabetes, and hypertension (Catalan et al., 2016; Cornier et al., 2008; Dandona, Aljada, Chaudhuri, Mohanty, & Garg, 2005).

It has been demonstrated in middle-aged overweight men that a phenolic-enriched supplementation with oleuropein and HT for 12 weeks, improves the glucose regulation and β-cell secretion capacity of insulin (de Bock et al., 2013).

Furthermore, it is well established that diabetes promotes an increase in lipid peroxidation (Gumieniczek, 2005), the alteration of the glutathione redox state, a decrease in plasmatic levels of antioxidants, and a reduction in the antioxidant enzyme activities. All these changes could produce oxidative stress caused by hyperglycaemia (Chaudhry, Ghosh, Roy, & Chandra, 2007). Some studies have demonstrated that a HT supplementation given to diabetic rats provoked a significant decrease in intestinal maltase, lactase, sucrose, and lipase activities and an enhancement of the SOD, CAT and GPx activities, as well as an increment in reduced glutathione levels, then reducing the oxidative stress status (Hamden et al., 2010). What is more, thiobarbituric acid reactive substances (TBARS) levels and the activity of lactate dehydrogenase (LDH) also decrease after HT administration (Hamden et al., 2010).

Moreover, HT improves plasma triacylglycerol and cholesterol levels and can decrease plasma low density lipoproteins (LDL), cholesterol and triacylglycerols, and it also increases HDL-cholesterol content (Hu, He, Jiang, & Xu, 2014).

Other authors (Hmimeda, Belarbia, & Visioli, 2016) observed that HT be able to decrease plasma hydroperoxides and augment the antioxidant status in rats with high-fat diet, but it could not decrease other oxidative markers as protein carbonyl. In addition, these authors discussed about the possibility that this antioxidant effect could be exerted as a consequence of the up-regulation of endogenous antioxidants mediated by Nrf-2 in response to harmful stimuli.

Further studies showed that HT could normalize obesity, diabetes, dyslipidaemia, inflammation, fatty liver, and insulin resistance induced by high fat diet in C57BL/6 mice (Cao et al., 2014) through down-regulating SREBP-1c/FAS pathway, decreasing oxidative stress, weakening mitochondrial anomalies, and suppressing apoptosis. In addition HT could decrease glucose levels and lipid metabolism present in db/db mice, as a confirmation the relevant effects of HT on metabolic syndrome (Cao et al., 2014). In this sense, HT also prevents of high glucose-induced generation of ROS and glucose-toxicity in SH-HY-5Y neuroblastoma cells (Zheng et al., 2015).

Altogether, these findings suggest that HT might be an effective agent for the prevention and treatment of diabetes and metabolic syndrome, but more clinical trial should be performed to determine by which mechanisms the HT can act against metabolic syndrome.

5.5. Anti-inflammatory effects of HT

HT is well established as a powerful phenolic antioxidant with potent anti-platelet, anti-inflammatory, and anti-atherogenic properties (Granados-Principal et al., 2010). A nutrigenomic study concluded that a high intake of phenol-enriched olive oils can repress the expression of several pro-inflammatory and pro-atherosclerotic genes, promoting a lower inflammatory profile in peripheral blood mononuclear cells (Camargo et al., 2010).

Studies carried out *in vitro* with human monocytic THP-1 cells treated with lipopolysaccharides to induce an inflammatory response, demonstrated that HT prevents from cytokines formation, nitric oxide (NO) generation, tumour necrosis factor-alpha (TNF-α) secretion and mRNA expression, as well as it inhibited iNOS and COX-2 expression (Zhang et al., 2009), showing a clear anti-inflammatory character of HT at cell level. On the other hand, in human monocytes the anti-inflammatory potential of HT was evaluated by assessing its effects on matrix metallopeptidase-9 (MMP-9) expression and its relation with COX-2/PGE2 pathway. HT inhibited MMP-9 gene and protein expression with a direct effect over the MMP-9 signalling pathway. The inhibitory effects of HT on MMP-9, COX-2, as well as on other pro-inflammatory genes depend on its inhibition of the protein kinase C (PKC) activation process (Scoditti et al., 2014).

Several studies performed (Carluccio et al., 2003; Killeen, Linder, Pontoniere, & Crea, 2014) showed that HT is able to modulate the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) transcription factor, related to more than 150 genes, including cytokines such as TNF-α, interleukin 1 (IL-1), IL-6 and IL-17, chemokines, and cell adhesion molecules, involved in inflammatory responses, thus contributing to explain in part the attenuation of vascular inflammation promoted by MD.

Some *in vivo* studies (Procopio et al., 2009) showed the capacity of HT to inhibit cyclooxygenase, COX-1 and COX-2 at the same level as in most consumed drugs such as ibuprofen and celecoxib. In patients with stabilized coronary disease, HT demonstrated its great ability to promote health-beneficial activities as an additional intervention to the pharmacological treatment, this fact was reached by decreasing IL6 and protein reactive C levels (Fito et al., 2008).

Richard et al. in 2011 described that HT is the most powerful anti-inflammatory (poly)phenol of olive oil. In this study, they observed a remarkable inhibition in the production of NO and prostaglandin E2 (PGE2), as well as a decrease in the secretion of cytokines such as IL-1α, IL-1β, IL-6, IL-12, TNF-α, and chemokines (CXCL10/IP-10, CCL2/MPC-1). The iNOS, IL-1α, CXCL10/IP-10, MIP-1β, MMP-9, and PGE2 synthase (PGES) gene expressions were also reduced by HT action.

All these findings indicate the great anti-inflammatory capacity of HT and how a daily intake of HT could modulate the inflammatory process and provides a health profit.

5.6. HT and respiratory diseases

Respiratory diseases are a complex group of different disorders that occur along the whole respiratory system, which comprises from the upper tract down to the pleural cavity, including both muscles and the nervous systems which modulates them. Due to this complexity, respiratory disorders can be derived from multiple causal agents such as pathogens, inflammation, air pollutants or our own individual genetics.

Visioli, Bellomo, and Galli (1998) demonstrated that HT and oleuropein can decrease the oxidative stress associated to neutrophils respiratory burst. Braga et al. (1997) also observed that HT could decrease the levels of superoxide anion in *in vitro* and *in vivo* models, and O'Dowd et al. (2004) determined that HT can scavenge hydrogen peroxide in a dose dependent manner during the respiratory burst when human neutrophils were stimulated. In addition, HT can inhibit the growth of several Gram-positive and Gram-negative bacteria related to respiratory tract infections (Bisignano et al., 1999). These finding contribute to consider to HT as a promising antimicrobial agent for treatment of respiratory tract infections.

A recent study of Liu, Fan, and Chen (2015) has showed that HT has some effect against pulmonary fibrosis in an induced-fibrosis model in rats. Adult Sprague Dawley rats were exposed to 22 Gy doses of radiations at a rate of 290 cGy/min to induce fibrosis. The mortality associated to HT was lower than then one with corticoid employed, and no congested oedema signs were observed. Not only that but HT also improved irradiation-induced regional fibrotic foci and collagen depositions of the lung, as well as it decreased the serum malondialdehyde concentration, increased the SOD activity, decreased the transforming growth factor β1 (TGF-β1), IL-6, IL-10, and TNF-α and activated nuclear transcription factors NF-E2-related factor 2 (Nrf-2). haem oxygenase-1 (HO-1) and NAD(P)H: quinone oxidoreductase-1 (NQO-1) protein. Besides, HT generated an inhibition of Alpha-actin-2 (marker of myofibroblast formation) expression and the promotion of surfactant-associated protein B expression (indispensable for lung function). All these results suggest that HT can decrease inflammation, fibrosis, and oxidative stress associated to pulmonary fibrosis with beneficial properties against respiratory pathologies.

5.7. Neuroprotection associated to HT

It is well know how high levels of oxidative and nitrosative stress can hurt the integrity and functioning of brain tissue, especially in aging. It is remarkable that HT presents a powerful capacity to scavenge radical species (Goya, Mateos, & Bravo, 2007) and to induce antioxidant enzymes (Martin et al., 2010) that could improve some neurodegenerative disorders such as Parkinson's disease (Dexter & Jenner, 2013).

In this sense, Schaffer et al. in 2007 founded that HT-rich extracts were able to attenuate the cytotoxic effects of Fe2 + and NOin murine-dissociated brain cells. Additionally, Schaffer et al. (2007) found that the oral administration of 100 mg of HT for 12 days in mice, improved the resistance of dissociated brain cells to oxidative stress, proved by reducing basal and stress-induced lipid peroxidation. Besides, these authors observed that basal mitochondrial membrane potential was also moderately hyperpolarized, showing for the first time in an *ex vivo* model that HT could exert a cytoprotectionat brain cells level.

Other studies in a hypoxia–reoxygenation model in brain slices from rats, showed that HT and HT-acetate had a neuroprotective effect (Gonzalez-Correa et al., 2008; Muñoz-Marin et al., 2012). Lactate dehydrogenase efflux in rat brain slices subjected to hypoxia and reoxygenation was significantly reduced under all experimental conditions and by a cytoprotective effect of HT, mediated by a reduction of oxidative and nitrosative stress, also in a hypoxia-reoxygention model.

Further studies (Tasset, Pontes, Hinojosa, de la Torre, & Tunez, 2011) showed that oral administration of EVOO and HT presented important effects on brain oxidative stress in a Huntington disease-like model performed on Wistar rats. Lipid peroxidation significantly decreased and cellular GSH increased as a mechanism of protection against oxidative damage by acting as brain antioxidants.

Some authors had determined that the neuroprotective role of HT was associated to its capacity to offset the oxidative damage, but more recently, Arunsundar, Shanmugarajan, and Ravichandran (2015) studied the potential effects of HT in C57BL/6 mice injected with oligomeric Aβ1–42 plus ibotenic acid to induce neuro-behavioural dysfunction. This injection produced an impairment of visuospatial and working memories skills. Spatio-cognitive performances significantly improved after 14 days of HT administration. These authors suggested that HT could down-regulate the expression of p38 and JNK, both closely linked to an impairment of the neuronal and memory functions (Sclip et al., 2011)

Finally, it has been described that maternal administration of HT to rats could restore the impaired neurogenesis and cognitive function caused by prenatal stress. In the study performed by Zheng et al. (Zheng

et al., 2015), rat's hippocampus showed that stress induced a down-regulation of neural proteins such as brain-derived neurotrophic factor (BDNF), growth associated protein 43 (GAP43), and synaptophysin, a low expression of glucocorticoid receptor, and a decrease in the antioxidant defence system (including Nrf2, SOD, and haem oxygenase 1 (HO-1)). All these changes finally lead to a detriment in the mitochondria content as well as in the ability to generate a proper antioxidant response in the hippocampus of offspring. The maternal supplementation with HT enhanced the mitochondrial content and modulated the activation of phase II enzymes, thus preventing from a cognitive impairment and biochemical alterations (Zheng et al., 2015).

5.8. HT and skin protection

Currently, dermatological conditions are taking great relevance due to the constant exposure to sunlight without protection factors, pollution or factors intrinsic to life itself as aging factors.

For a couple of decades, several studies have determined how the MD, and particularly the OO, can reduce the incidence of skin tumours among other health-beneficial aspects (Owen et al., 2000). Some authors have employed different olive (poly)phenol-based nutraceuticals to determine that part of the skin protector effects associated to the MD can be attributable to (poly)-phenols from MD and particularly from OO. In this sense, Herrera Acosta, Alonso Suárez Pérez, Aguilera Arjona, and Visioli (2016) showed the antipsoriasic-beneficial properties of a (poly)phenol-based cosmeceutical (Alyvium®) in humans. They speculated about the possibility that the positive skin effects of Alyvium® could be determined as consequence of its role in the normalization of the redox code, improving the cutaneous manifestation.

It is well known that ultraviolet (UV) radiation promotes skin damage by generating ROS, a depletion of endogenous antioxidant system (Park, Kim, Hong, Kim, & Oh, 2014) and double-stranded DNA breakage. The increment in oxidative stress produces damage to skin cells, provoking dermatological disorders (Cooke, Evans, Dizdaroglu, & Lunec, 2003). Some studies have demonstrated that HT and its methylated metabolites can decrease, in a dose dependent manner, the oxidative stress associated to UV radiation and can also provoke a cytotoxic effect in melanoma cells (M14) exposed to UVA radiation (D'Angelo et al., 2005). Similar skin-protective results were obtained in a human skin immortalized keratinocyte cell line (HaCaT) where HT had a significant shielding capacity against UVB negative effects (Guo, An, Jiang, Geng, & Zhong, 2010; Salucci et al., 2014). In addition, a novel HT dimeric derivative also showed a high capacity against UV-derived oxidative stress in HaCaT cells (Zwane et al., 2012) due to its radical scavenging activity. This dimeric-HT also showed the role in apoptosis by modulation of Bcl-2 and Bax proteins.

5.9. HT as antitumour compound

Firstly, it should be noted that ROS are directly linked to tumour formation, mainly due to their key role in the initiation, promotion and progression stages of cancer development. For that reason, the antitumour properties of HT will be mostly associated to its activity as ROS scavenger or its antioxidant system modulation capacity (Fabiani, 2016; López de Las Hazas, Piñol, Macià, & Motilva, 2017). However, in the last decade, a wide number of studies have been performed in order to demonstrate exclusively the antitumour effect of HT.

In human hepatoma cell line (HepG2) acrylamide-induced genotoxicity model, HT can have a chemo modulator effect against genotoxicity by decreasing the ROS production provoked by acrylamide (Zhang, Jiang, Geng, Yoshimura, & Zhong, 2008). Thanks to preliminary studies, a dose around 10–17 mg/g of HT has been established to start showing antitumour effects (Quirantes-Pine et al., 2013).

In addition, numerous studies have tried to explain how HT can act as an antitumour compound and tried to elucidate its antiproliferative action on certain types of tumours, independently of its modulating ability over the antioxidant system (Terzuoli, Giachetti, Ziche, & Donnini, 2016).

HT may promote apoptosis and it inhibits proliferation and growth in different types of tumour cells, such as HL60 (promyelocytic leukemia), HT29 and HT29 clone 19A (colon adenocarcinoma) by arresting G0/G1 phase of cell cycle, with a consequent decrease in the cell percentage in the S and G2/M phases (Fabiani et al., 2002). A study carried out by Fabiani, Rosignoli, De Bartolomeo, Fuccelli, and Morozzi (2008), corroborated the effects of HT on these cells, suggesting a possible mechanism through an increment of cyclin D3, CDKi p21WAF1/Cip1 and p27Kip1, and a decrease of CDK6. All these changes would be involved in the inhibition of the G1-S transition and in the regulation of differentiation and apoptosis processes of HL60 cells.

In thyroid cancer cell lines [papillary (TPC-1, FB-2) and follicular (WRO)], HT has also demonstrated its abilities to decrease cell viability and also to activate apoptosis through mitochondria path (Toteda et al., 2017).

On the other hand, there are many studies carried out in several types of healthy and normal cells which have demonstrated non-toxicity and chemo protective effects of HT in non-tumour cell cultures (Fabiani et al., 2002; Rosignoli, Fuccelli, Sepporta, & Fabiani, 2016; Warleta et al., 2011). One of the mechanisms of HT over the apoptotic process is related to the activation of c-jun by NH2-cjun-kinase which provoke cellular death and inactivation of Bcl-2 (B-cell lymphoma 2) protein (Della Ragione et al., 2002; Granados-Principal et al., 2014).

Related to a UV-A irradiated human melanoma cells (M14). D'Angelo et al. in 2005 demonstrated that HT could protect these injured cells in a dose dependent manner and could also act as a cell cycle inhibitor at dosages upper to 400 μM.

To sum up, two different mechanisms of action have been proposed by Granados-Principal et al. (2010) for HT's antitumour effect:

Blockade of Cyclin-dependent kinases (*CDKs*). This activity can be performed by exerting both a direct blockade of CDKs and by an induction of CDKs inhibitors.

Blocking messengers involved in cell proliferation, such as ROS.

Moreover, the study carried out by Guichard et al. (2006), showed that HT induced cell death by apoptosis and promoting a prolonged endoplasmic reticulum (ER) stress in human colon carcinoma HT-29 cells, disrupting ER homeostasis and activating “unfolded protein response” signalling pathways. HT also promoted S and G2/M phases arrest preventing cells from entering into mitosis, reaching around 65% of cell death, and triggered proteolytic cleavage of the pro-caspase 3. Finally, in the same study, HT prevented the activation of TNF-α-dependent NF-kβ by inhibiting the pro-survival pathway phosphoinositol 3 kinase/v-akt murine thymoma viral oncogene/PKB. Besides what is mentioned above, Sun et al. in 2014 demonstrated that HT presents a selective anti-proliferative effect on human colon cancer cells (DLD1 cells) by promoting oxidative stress. According to Sun et al. (2014), HT is able to activate the phosphoinositide 3-kinase/Akt pathway, phosphorylates FOXO3a and then downregulates FOXO3a's target genes with no cytotoxic effects in normal colon epithelial cells.

Thanks to the intense first phase of metabolism that HT supports in the bowel by microflora, several studies tried to determine if HT's catabolites microbiota-derived had also the antitumour activity associated to HT alone (Bernini et al., 2017). In this sense, Lopez de las Hazas et al. in 2017 could observed the antiproliferative and pro-apoptotic properties of both HT and its derivatives in colonic cancer cells (HT-29 and Caco2 cell lines).

Likewise, Cárdeno, Sanchez-Hidalgo, Rosillo, & Alarcon de la Lastra in 2013, proposed that HT can also lead to a growth inhibition in HT-29 by decreasing HIF-1α protein levels and upregulating p53 protein expressions.

In addition, HT has also shown an antiproliferative effect in human colon adenocarcinoma cells (Caco-2) because of its powerful inhibition of extracellular signal-regulated kinase (ERK)1/2 phosphorylation and

because of the reduction of cyclin D1 expression (Corona et al., 2009).

On the other hand, fatty acid synthase (FASN) catalyses the formation of fatty acids from acetyl-CoA, malonyl-CoA, and NADPH with a key role in lipid biosynthesis. Many types of human tumours [colorectal cancer (Rashid et al., 1997), breast cancer (Menendez & Lupu, 2006) or prostate and ovarian cancers (Wang, Kuhajda, Sokoll, & Chan, 2001)] present increased levels of FASN type I. The FASN role is still unclear although inhibitors of FASN have been employed for its high ability to promote cell proliferation reduction (Mullen & Yet, 2015). Notarnicola et al. (2011), performed a study with HT and human colorectal cancer cells (SW620). In this study, they observed that HT inhibited FANS in SW620 cells, but no in HT-29, suggesting that a lower grade of cell differentiation makes some cells more sensitive to apoptosis induced by HT. This mechanism of FASN inhibition is also shared with human hepatoma HepG2 and Hep3B cell lines (Tutino, Caruso, Messa, Perri, & Notarnicola, 2012).

In relation to breast cancer, HT is able to inhibit Human epidermal growth factor receptor 2 (HER2) in breast cancer cells trastuzumab resistant with an overexpressed HER2 oncogene (SkBr3). Besides HT promotes cytotoxicity in MCF7 cells which naturally express HER2 (Menendez et al., 2007).

Another proposed mechanisms of action of HT in MCF-7 is the inhibition of cell proliferation rate and the induction of cell apoptosis, as well as the blockage of G(1) to S phase transition, increasing the number of cells in G(0)/G(1) phase at 50 μg/ml (Han, Talorete, Yamada, & Isoda, 2009). A further study determined that MCF-7 cells subjected to the action of a HT enrich-extract, suffered a cell cycle arrest in G0/G1 phase, due to a down-expression of the peptidyl-prolyl cis-trans isomerase Pin1, which decrease Cyclin D1 (over-expressed in 50% of breast cancer patients) and to an overexpression of c-jun (Bouallagui, Han, Isoda, & Sayadi, 2011). The study carried out by Corona et al. with CaCo2 cells (Corona et al., 2009), observed how HT altered the phosphorylation status of the extracellular signal-related kinase 1 and 2 (ERK1/2), inhibiting the activation of ERK1/2 with the subsequent decrease in Cyclin D1. Like what happened in the study performed by Chimento et al. in 2010. These authors determined that HT also inhibits the activation of ERK1/2-estrogen-dependent proliferation pathway in MCF7 cells, performing its chemo-protective and antiproliferative roles through non-traditional estrogen-dependent rapid signals.

Further studies showed that HT decreased tumour volume and growth, and cell proliferation in Sprague-Dawley rats with breast tumours induced by DMBA. Another possible mechanism of action was then proposed *via* Wnt pathway. HT promotes an induction in the synthesis of Sfrp4 (Secreted frizzled-related protein 4), which in turn acts as a soluble modulator of Wnt pathway, facilitating apoptosis and blocking cell proliferation in breast cancer (Granados-Principal et al., 2011).

Finally, several epidemiological studies have shown that both HT and its derivatives present in olive oil may have protective effects against multiple types of cancer such as pancreas, oral cavity, oesophagus, colon-rectum, prostate or lung (Rafehi et al., 2012; Vilaplana-Perez, Aunon, Garcia-Flores, & Gil-Izquierdo, 2014).

6. Conclusions

According to all data revised in this review we can elucidate why a compound from olive oil has gained over the years such great importance in terms of health protection. Different processes suffered by HT which are related to the bioavailability along the body and its relationship with different endogenous pathways have been also been described in details. At this point, it should be noted how the discovery of these modifications have served to the industry to redirect the application of this compound, generating new derived molecules from HT allowing to improve the bioavailability, activity and power.

We also reviewed a wide variety of health-beneficial effects attributable to HT such as a health protector and in many cases as a result of applying novel dosage forms that improved the characteristics of this molecule and its derivatives.

Finally, we must emphasize the antioxidant/prooxidant duality of HT, being the first its most important role and for which it is capable of performing most of its healthy activities. It is also noticeable its selective effect as prooxidant in certain tumour cells, exercising an antitumour action as a set of two perfectly coordinated effects.

Conflict of interest

Authors declare no conflict of interest or any financial benefit from direct or indirect applications of the research.

Author contributions

All authors have participated actively in the conception and design of the review. They also have established different points of view about the molecule studied and its role in health, being inescapable part in this study. Cesar Ramirez-Tortosa, Carmen Rodriguez-Garcia, José Luis Quiles and M. Carmen Ramirez-Tortosa have also participated in the paper revision and interpretation, as well as drafting. Maria Robles-Almazan, Mario Pulido-Moran, and Jorge Moreno-Fernandez have written different sections of this paper and have actively and equally participated in the revision and conceptualization of this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Alarcón Flores, M. I., Romero-González, R., Garrido Frenich, A., & Martínez Vidal, J. L. (2012). Analysis of phenolic compounds in olive oil by solid-phase extraction and ultra high performance liquid chromatography–tandem mass spectrometry. *Food Chemistry, 134*(4), 2465–2472.

Anter, J., Tasset, I., Demyda-Peyras, S., Ranchal, I., Moreno-Millan, M., Romero-Jimenez, M., ... Alonso-Moraga, A. (2014). Evaluation of potential antigenotoxic, cytotoxic and proapoptotic effects of the olive oil by-product “alperujo”, hydroxytyrosol, tyrosol and verbascoside. *Mutation Research, Genetic Toxicology and Environmental Mutagenesis, 772*, 25–33.

Arunsundar, M., Shanmugarajan, T., & Ravichandran, V. (2015). 3,4-dihydroxyphenylethanol attenuates spatio-cognitive deficits in an Alzheimer's disease mouse model: Modulation of the molecular signals in neuronal survival-apoptotic programs. *Neurotoxicity Research, 27*(2), 143–155.

Atzeri, A., Lucas, R., Incani, A., Peñalver, P., Zafra-Gómez, A., Melis, M. P., ... Deiana, M. (2016). Hydroxytyrosol and tyrosol sulfate metabolites protect against the oxidized cholesterol pro-oxidant effect in Caco-2 human enterocyte-like cells. *Food & Function, 7*(1), 337–346.

Aunon-Calles, D., Canut, L., & Visioli, F. (2013). Toxicological evaluation of pure hydroxytyrosol. *Food and Chemical Toxicology, 55*, 498–504.

Aunon-Calles, D., Giordano, E., Bohnenberger, S., & Visioli, F. (2013). Hydroxytyrosol is not genotoxic in vitro. *Pharmacological Research, 74*, 87–93.

Babich, H., & Visioli, F. (2003). In vitro cytotoxicity to human cells in culture of some phenolics from olive oil. *Farmaco, 58*, 403–407.

Bai, C., Yan, X., Takenaka, M., Sekiya, K., & Nagata, T. (1998). Determination of synthetic hydroxytyrosol in rat plasma by GC–MS. *Journal of Agricultural and Food Chemistry, 46*(10), 3998–4001.

Bayram, B., Ozcelik, B., Grimm, S., Roeder, T., Schrader, C., Ernst, I. M. A., ... Rimbach, G. (2012). A diet rich in olive oil phenolics reduces oxidative stress in the heart of SAMP8 mice by induction of Nrf2-dependent gene expression. *Rejuvenation Research, 15*(1), 71–81.

Bernini, R., Carastro, I., Palmini, G., Tanini, A., Zonefrati, R., Pinelli, P., ... Romani, A. (2017). Lipophilization of hydroxytyrosol-enriched fractions from *Olea europaea* L. byproducts and evaluation of the in vitro effects on a model of colorectal cancer cells. *Journal of Agricultural and Food Chemistry, 22*. http://dx.doi.org/10.1021/acs.jafc.6b05457.

Bernini, R., Gilardini, R., Montani, M. S., Merendino, N., Romani, A., & Velotti, F. (2015). Hydroxytyrosol-derived compounds: A basis for the creation of new pharmacological agents for cancer prevention and therapy. *Journal of Medicinal Chemistry, 58*(23), 9089–9107.

Bernini, R., Merendino, N., Romani, A., & Velotti, F. (2013). Naturally occurring hydroxytyrosol: Synthesis and anticancer potential. *Current Medicinal Chemistry, 20*(5), 655–670.

Bisignano, G., Tomaino, A., Lo Cascio, R., Crisafi, G., Uccella, N., & Saija, A. (1999). On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *The Journal of Pharmacy and Pharmacology, 51*(8), 971–974.
Bjelakovic, G., Nikolova, D., & Gluud, C. (2014). Antioxidant supplements and mortality. *Current Opinion in Clinical Nutrition and Metabolic Care, 17*(1), 40–44.
Boskou, D. (2008). *Olive oil: Minor constituents and health.* CRC Press.
Bouallagui, Z., Han, J., Isoda, H., & Sayadi, S. (2011). Hydroxytyrosol rich extract from olive leaves modulates cell cycle progression in MCF-7 human breast cancer cells. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 49*(1), 179–184.
Braga, P. C., Mancini, L., Guffanti, E. E., Dal Sasso, M., Sala, M., & Reggio, S. (1997). Effects of nedocromil sodium on the oxidative burst of polymorphonuclear leukocytes: Comparison with salbutamol. *Drugs Under Experimental and Clinical Research, 23*(1), 33–38.
Briante, R., La Cara, F., Febbraio, F., Patumi, M., & Nucci, R. (2002). Bioactive derivatives from oleuropein by a biotransformation on *Olea europaea* leaf extracts. *Journal of Biotechnology, 93*(2), 109–119.
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*(17), 4934–4940.
Buckland, G., & Gonzalez, C. A. (2015). The role of olive oil in disease prevention: A focus on the recent epidemiological evidence from cohort studies and dietary intervention trials. *The British Journal of Nutrition, 113*(2), S94–101.
Buettner, G. R., Ng, C. F., Wang, M., Rodgers, V., & Schafer, F. Q. (2006). A new paradigm: Manganese superoxide dismutase influences the production of H2O2 in cells and thereby their biological state. *Free Radical Biology & Medicine, 41*(8), 1338–1350.
Burattini, S., Salucci, S., Baldassarri, V., Accorsi, A., Piatti, E., Madrona, A., ... Falcieri, E. (2013). Anti-apoptotic activity of hydroxytyrosol and hydroxytyrosyl laurate. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 55*, 248–256.
Calderon-Montano, J. M., Madrona, A., Burgos-Moron, E., Orta, M. L., Mateos, S., Espartero, J. L., & Lopez-Lazaro, M. (2013). Selective cytotoxic activity of new lipophilic hydroxytyrosol alkyl ether derivatives. *Journal of Agricultural and Food Chemistry, 61*(21), 5046–5053.
Camargo, A., Ruano, J., Fernandez, J. M., Parnell, L. D., Jimenez, A., Santos-Gonzalez, M., ... Perez-Jimenez, F. (2010). Gene expression changes in mononuclear cells in patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil. *BMC Genomics, 11*, 253.
Cao, K., Xu, J., Zou, X., Li, Y., Chen, C., Zheng, A., ... Feng, Z. (2014). Hydroxytyrosol prevents diet-induced metabolic syndrome and attenuates mitochondrial abnormalities in obese mice. *Free Radical Biology & Medicine, 67*, 396–407.
Carluccio, M. A., Siculella, L., Ancora, M. A., Massaro, M., Scoditti, E., Storelli, C., ... De Caterina, R. (2003). Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: Antiatherogenic properties of Mediterranean diet phytochemicals. *Arteriosclerosis, Thrombosis, and Vascular Biology, 23*(4), 622–629.
Catalan, U., Lopez de Las Hazas, M. C., Rubio, L., Fernandez-Castillejo, S., Pedret, A., de la Torre, R., ... Sola, R. (2015). Protective effect of hydroxytyrosol and its predominant plasmatic human metabolites against endothelial dysfunction in human aortic endothelial cells. *Molecular Nutrition & Food Research, 59*, 2523–2536.
Catalan, U., Rubio, L., Lopez de Las Hazas, M. C., Herrero, P., Nadal, P., Canela, N., ... Sola, R. (2016). Hydroxytyrosol and its complex forms (secoiridoids) modulate aorta and heart proteome in healthy rats: Potential cardio-protective effects. *Molecular Nutrition & Food Research, 60*(10), 2114–2129.
Charoenprasert, S., & Mitchell, A. (2012). Factors influencing phenolic compounds in table olives (*Olea europaea*). *Journal of Agricultural and Food Chemistry, 60*(29), 7081–7095.
Chashmi, N. A., Emadi, S., & Khastar, H. (2017). Protective effects of hydroxytyrosol on gentamicin induced nephrotoxicity in mice. *Biochemical and Biophysical Research Communications, 482*(4), 1427–1429.
Chaudhry, J., Ghosh, N. N., Roy, K., & Chandra, R. (2007). Antihyperglycemic effect of a new thiazolidinedione analogue and its role in ameliorating oxidative stress in alloxan-induced diabetic rats. *Life Sciences, 80*(12), 1135–1142.
Christian, M. S., Sharper, V. A., Hoberman, A. M., Seng, J. E., Fu, L., Covell, D., ... Crea, R. (2004). The toxicity profile of hydrolyzed aqueous olive pulp extract. *Drug and Chemical Toxicology, 27*(4), 309–330.
Cicerale, S., Conlan, X. A., Sinclair, A. J., & Keast, R. S. J. (2009). Chemistry and health of olive oil phenolics. *Critical Reviews in Food Science and Nutrition, 49*(3), 218–236.
Cooke, M. S., Evans, M. D., Dizdaroglu, M., & Lunec, J. (2003). Oxidative DNA damage: Mechanisms mutation and disease. *FASEB Journal: official publication of the Federation of American Societies for Experimental Biology, 17*(10), 1195–1214.
Cornier, M. A., Dabelea, D., Hernandez, T. L., Lindstrom, R. C., Steig, A. J., Stob, N. R., ... Eckel, R. H. (2008). The metabolic syndrome. *Endocrine Reviews, 29*(7), 777–822.
Corona, G., Deiana, M., Incani, A., Vauzour, D., Dessi, M. A., & Spencer, J. P. E. (2009). Hydroxytyrosol inhibits the proliferation of human colon adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. *Molecular Nutrition & Food Research, 53*(7), 897–903.
Couto, E., Boffetta, P., Lagiou, P., Ferrari, P., Buckland, G., Overvad, K., ... Trichopoulou, A. (2011). Mediterranean dietary pattern and cancer risk in the EPIC cohort. *British Journal of Cancer, 104*(9), 1493–1499.
Covas, M. I., de la Torre, R., & Fito, M. (2015). Virgin olive oil: A key food for cardiovascular risk protection. *The British Journal of Nutrition, 13*, S19–28.
Crespo, M. C., Tomé-Carneiro, J., Burgos-Ramos, E., Loria Kohen, V., Espinosa, M. I., Herranza, J., & Visioli, F. (2015). One-week administration of hydroxytyrosol to humans does not activate phase II enzymes. *Pharmacological Research, 95-96*, 132–137.
Crisante, F., Taresco, V., Donelli, G., Vuotto, C., Martinelli, A., D'Ilario, L., ... Piozzi, A. (2015). Antioxidant Hydroxytyrosol-based Polyacrylate with antimicrobial and antiadhesive activity versus staphylococcus epidermidis. *Advances in Experimental Medicine and Biology, 901*, 25–36.
Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., & Garg, R. (2005). Metabolic syndrome: A comprehensive perspective based on interactions between obesity diabetes and inflammation. *Circulation, 111*(11), 1448–1454.
D'Angelo, S., Ingrosso, D., Migliardi, V., Sorrentino, A., Donnarumma, G., Baroni, A., & Galletti, P. (2005). Hydroxytyrosol a natural antioxidant from olive oil prevents protein damage induced by long-wave ultraviolet radiation in melanoma cells. *Free Radical Biology & Medicine, 38*(7), 908–919.
D'Angelo, S., Manna, C., Migliardi, V., Mazzoni, O., Morrica, P., Capasso, G., ... Zappia, V. (2001). Pharmacokinetics and metabolism of hydroxytyrosol a natural antioxidant from olive oil. *Drug Metabolism and Disposition: The Biological Fate of Chemicals, 29*(11), 1492–1498.
de Bock, M., Derraik, J. G. B., Brennan, C. M., Biggs, J. B., Morgan, P. E., Hodgkinson, S., ... Cutfiel, W. S. (2013). Olive (Olea Europaea L,) leaf polyphenols improve insulin sensitivity in middle-aged overweight men: A randomized placebo-controlled crossover trial. *PLoS One, 8*(3), e57622.
de Bock, M., Thorstensen, E. B., Derraik, J. G. B., Henderson, H. V., Hofman, P. L., & Cutfield, W. S. (2013). Human absorption and metabolism of oleuropein and hydroxytyrosol ingested as olive (Olea Europaea L,) leaf extract. *Molecular Nutrition & Food Research, 57*(11), 2079–2085.
de la Torre, R., Covas, M. I., Pujadas, M. A., Fito, M., & Farre, M. (2006). Is dopamine behind the health benefits of red wine? *European Journal of Nutrition, 45*(5), 307–310.
de Roos, B., Zhang, X., Rodriguez- Gutierrez, G., Wood, S., Rucklidge, G. J., Reid, M. D., ... O'Kennedy, N. (2011). Anti-platelet effects of olive oil extract: In vitro functional and proteomic studies. *European Journal of Nutrition, 50*(7), 553–562.
Deiana, M., Aruoma, O. I., Bianchi, M. L., Spencer, J. P., Kaur, H., Halliwell, B., ... Corongiu, F. P. (1999). Inhibition of peroxynitrite dependent DNA base modification and tyrosine nitration by the extra virgin olive oil-derived antioxidant hydroxytyrosol. *Free Radical Biology & Medicine, 26*(5–6), 762–769.
Della Ragione, F., Cucciolla, V., Criniti, V., Indaco, S., Borriello, A., & Zappia, V. (2002). Antioxidants induce different phenotypes by a distinct modulation of signal transduction. *FEBS Letters, 532*(3), 289–294.
Dexter, D. T., & Jenner, P. (2013). Parkinson disease: From pathology to molecular disease mechanisms. *Free Radical Biology and Medicine, 62*, 132–144.
Di Tommaso, D., Calabrese, R., & Rotilio, D. (1998). Identification and quantitation of Hydroxytyrosol in Italian wines. *Journal of High Resolution Chromatography, 21*(10), 549–553.
Diamanti, J., Mezzetti, B., Giampieri, F., Alvarez-Suarez, J. M., Quiles, J. L., Gonzalez-Alonso, A., et al. (2014). Doxorubicin-induced oxidative stress in rats is efficiently counteracted by dietary anthocyanin differently enriched strawberry (*Fragaria* × *ananassa* Duch). *Journal of Agricultural and Food Chemistry, 62*(18), 3935–3943.
Dominguez-Perles, R., Aunon, D., Ferreres, F., & Gil-Izquierdo, A. (2017). Gender differences in plasma and urine metabolites from Sprague-Dawley rats after oral administration of normal and high doses of hydroxytyrosol hydroxytyrosol acetate and DOPAC. *European Journal of Nutrition, 56*(1), 215–224.
Estruch, R., Martinez-Gonzalez, M. A., Corella, D., Salas-Salvado, J., Ruiz-Gutierrez, V., Covas, M. I., ... Ros, E. (2006). Effects of a Mediterranean-style diet on cardiovascular risk factors: A randomized trial. *Annals of Internal Medicine, 145*(1), 1–11.
Fabiani, R. (2016). Anti-cancer properties of olive oil secoiridoid phenols: A systematic review of in vivo studies. *Food & Function, 12*, 4145–4159.
Fabiani, R., De Bartolomeo, A., Rosignoli, P., Servili, M., Montedoro, G. F., & Morozzi, G. (2002). Cancer chemoprevention by hydroxytyrosol isolated from virgin olive oil through G1 cell cycle arrest and apoptosis. *European Journal of Cancer Prevention: the official journal of the European Cancer Prevention Organisation (ECP), 11*(4), 351–358.
Fabiani, R., De Bartolomeo, A., Rosignoli, P., Servili, M., Selvaggini, R., Montedoro, G. F., ... Morozzi, G. (2006). Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differentiation. *Journal of Nutrition, 136*, 614–619.
Fabiani, R., Rosignoli, P., De Bartolomeo, A., Fuccelli, R., & Morozzi, G. (2008). Inhibition of cell cycle progression by hydroxytyrosol is associated with upregulation of cyclin-dependent protein kinase inhibitors p21(WAF1/Cip1) and p27(Kip1) and with induction of differentiation in HL60 cells. *The Journal of Nutrition, 138*(1), 42–48.
Fabrizio, P., Pletcher, S. D., Minois, N., Vaupel, J. W., & Longo, V. D. (2004). Chronological aging-independent replicative life span regulation by Msn2/Msn4 and Sod2 in Saccharomyces Cerevisiae. *FEBS Letters, 557*(1–3), 136–142.
Fernandez-Avila, C., Montes, R., Castellote, A. I., Chisaguano, A. M., Fito, M., Covas, M. I., ... Lopez-Sabatel, M. C. (2015). Fast determination of virgin olive oil phenolic metabolites in human high-density lipoproteins. *Biomedical Chromatography: BMC, 29*(7), 1035–1041.
Fernández-Mar, M. I., Mateos, R., García-Parrilla, M. C., Puertas, B., & Cantos-Villar, E. (2012). Bioactive compounds in wine: Resveratrol hydroxytyrosol and melatonin: A review. *Food Chemistry, 130*(4), 797–813.
Fito, M., Cladellas, M., de la Torre, R., Marti, J., Munoz, D., Schroder, H., ... Covas, M. I. (2008). Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: A randomized crossover controlled trial. *European Journal of Clinical Nutrition, 62*(4), 570–574.
Forman, H. J., Davies, K. J., & Ursini, F. (2014). How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radical Biology & Medicine, 66*, 24–35.
García, A., Brenes, M., Romero, C., García, P., & Garrido, A. (2002). Study of phenolic compounds in virgin olive oils of the Picual variety. *European Food Research and Technology, 215*, 407–412.
García-Villalba, R., Carrasco-Pancorbo, A., Oliveras-Ferraros, C., Vázquez-Martín, A.,

Menéndez, J. A., Segura-Carretero, A., & Fernández-Gutiérrez, A. (2010). Characterization and quantification of phenolic compounds of extra-virgin olive oils with anticancer properties by a rapid and resolutive LC-ESI-TOF MS method. *Journal of Pharmaceutical and Biomedical Analysis, 52*(2), 416–429.

Godoy-Caballero, M. P., Acedo-Valenzuela, M. I., & Galeano-Diaz, T. (2012). Simple quantification of phenolic compounds present in the minor fraction of virgin olive oil by LC-DAD-FLD. *Talanta, 101*, 479–487.

Goncalves, I., Andersson-Georgiadou, E., Mattsson, S., Skog, G., Pedro, L., Fernandes, E. F., ... Strenstom, K. (2015). Direct association between diet and the stability of human atherosclerotic plaque. *Scientific Reports, 5*, 15524.

Gonzalez-Alonso, A., Perez-Lopez, P., Varela-Lopez, A., Ramirez-Tortosa, M. C., Battino, M., & Quiles, J. L. (2015). Experimental evidence on the role of different types unsaturated fats in the diet on ageing. *Revista Española De Geriatrias Y Gerontologia, 50*(6), 285–288.

Gonzalez-Correa, J. A., Navas, M. D., Lopez-Villodres, J. A., Trujillo, M., Espartero, J. L., & De La Cruz, J. P. (2008). Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia-reoxygenation. *Neuroscience Letters, 446*(2–3), 143–146.

Gonzalez-Santiago, M., Fonolla, J., & Lopez-Huertas, E. (2010). Human absorption of a supplement containing purified hydroxytyrosol a natural antioxidant from olive oil and evidence for its transient association with low-density lipoproteins. *Pharmacological Research, 61*(4), 364–370.

Gonzalez-Santiago, M., Martin-Bautista, E., Carrero, J. J., Fonolla, J., Baro, L., Bartolome, M. V., et al. (2006). One-month administration of hydroxytyrosol a phenolic antioxidant present in olive oil to hyperlipemic rabbits improves blood lipid profile antioxidant status and reduces atherosclerosis development. *Atherosclerosis, 188*(1), 35–42.

Goya, L., Mateos, R., & Bravo, L. (2007). Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells, protection against oxidative stress induced by tert-butylhydroperoxide. *European Journal of Nutrition, 46*(2), 70–78.

Granados-Principal, S., El-Azem, N., Pamplona, R., Ramirez-Tortosa, C., Pulido-Moran, M., Vera-Ramirez, L., & Ramirez-Tortosa, M. C. (2014). Hydroxytyrosol ameliorates oxidative stress and mitochondrial dysfunction in doxorubicin-induced cardiotoxicity in rats with breast cancer. *Biochemical Pharmacology, 90*(1), 25–33.

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C., Camacho-Corencia, P., Sanchez-Rovira, P., Vera-Ramirez, L., & Ramirez-Tortosa, M. C. (2011). Hydroxytyrosol inhibits growth and cell proliferation and promotes high expression of sfrp4 in rat mammary tumours. *Molecular Nutrition & Food Research, 55*, S117–126.

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C. L., Ochoa-Herrera, J., Perez-Lopez, P., Pulido-Moran, M., & Ramirez-Tortosa, M. C. (2012). Squalene ameliorates atherosclerotic lesions through the reduction of CD36 scavenger receptor expression in macrophages. *Molecular Nutrition & Food Research, 56*(5), 733–740.

Granados-Principal, S., Quiles, J. L., Ramirez-Tortosa, C. L., Sanchez-Rovira, P., & Ramirez-Tortosa, M. C. (2010). Hydroxytyrosol: From laboratory investigations to future clinical trials. *Nutrition Reviews, 68*(4), 191–206.

Guichard, C., Pedruzzi, E., Fay, M., Marie, J. C., Braut-Boucher, F., Daniel, F., Grodet, A., ... Ogier-Denis, E. (2006). Dihydroxyphenylethanol induces apoptosis by activating serine/threonine protein phosphatase PP2A and promotes the endoplasmic reticulum stress response in human colon carcinoma cells. *Carcinogenesis, 27*(9), 1812–1827.

Gumieniczek, A. (2005). Effects of pioglitazone on hyperglycemia-induced alterations in antioxidative system in tissues of alloxan-treated diabetic animals. *Experimental and Toxicologic Pathology, 56*(4–5), 321–326.

Guo, W., An, Y., Jiang, L., Geng, C., & Zhong, L. (2010). The protective effects of hydroxytyrosol against UVB-induced DNA damage in HaCaT cells. *Phytotherapy Research: PTR, 24*(3), 352–359.

Hamden, K., Allouche, N., Jouadi, B., El-Fazaa, S., Gharbi, N., Carreau, S., ... Elfeki, A. (2010). Inhibitory action of purified hydroxytyrosol from stored olive mill waste on intestinal disaccharidases and lipase activities and pancreatic toxicity in diabetic rats. *Food Science and Biotechnology, 19*(2), 439–447.

Han, J., Talorete, T. P. N., Yamada, P., & Isoda, H. (2009). Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. *Cytotechnology, 59*(1), 45–53.

Hashimoto, T., Ibi, M., Matsuno, K., Nakashima, S., Tanigawa, T., Yoshikawa, T., & Yabe-Nishimura, C. (2004). An endogenous metabolite of dopamine 34-dihydroxyphenylethanol acts as a unique cytoprotective agent against oxidative stress-induced injury. *Free Radical Biology & Medicine, 36*(5), 555–564.

Heilman, J., Anyangwe, N., Tran, N., Edwards, J., Beilstein, P., & Lopez, J. (2015). Toxicological evaluation of an olive extract H35: Subchronic toxicity in the rat. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 84*, 18–28.

Herrera Acosta, E., Alonso Suárez Pérez, J., Aguilera Arjona, J., & Visioli, F. (2016). An olive polyphenol-based nutraceutical improves cutaneous manifestations of psoriasis in humans. *PharmaNutrition, 4*, 151–153.

Hmimeda, S., Belarbia, M., & Visioli, F. (2016). Hydroxytyrosol augments the redox status of high fat diet-fed rats. *PharmaNutrition, 4*, 139–142.

Hu, T., He, X. W., Jiang, J. G., & Xu, X. L. (2014). Hydroxytyrosol and its potential therapeutic effects. *Journal of Agricultural and Food Chemistry, 62*(7), 1449–1455.

Jemai, H., Bouaziz, M., Fki, I., El Feki, A., & Sayadi, S. (2008). Hypolipidimic and antioxidant activities of oleuropein and its hydrolysis derivative-rich extracts from Chemlali olive leaves. *Chemico-Biological Interactions, 176*(2–3), 88–98.

Kedechi, S., Zribi, N., Louati, N., Menif, H., Sellami, A., Lassoued, S., ... Chackroun, N. (2016). Antioxidant effect of hydroxytyrosol on human sperm quality during in vitro incubation. *Andrologia, Feb, 49*(1), http://dx.doi.org/10.1111/and.12595 (7).

Khayyal, M. T., el-Ghazaly, M. A., Abdallah, D. M., Nassar, N. N., Okpanyi, S. N., & Kreuter, M. H. (2002). Blood pressure lowering effect of an olive leaf extract (*Olea europaea*) in L-NAME induced hypertension in rats. *Arzneimittel-Forschung, 52*(11), 797–802.

Khymenets, O., Crespo, C., Dangles, O., Rakotomanomana, N., Andres-Lacueva, C., & Visioli, F. (2016). Human hydroxytyrosol's absorption and excretion from a nutraceutical. *Journal of Functional Foods, 23*, 278–282.

Khymenets, O., Fitó, M., Covas, M. I., Farré, M., Pujadas, M. A., Muñoz, D., ... de la Torre, R. (2009). Mononuclear cell transcriptome response after sustained virgin olive oil consumption in humans: An exploratory nutrigenomics study. *OMICS A Journal of Integrative Biology, 13*(1), 7–19.

Khymenets, O., Fitó, M., Touriño, S., Muñoz-Aguayo, D., Pujadas, M., Torres, J. L., ... de la Torre, R. (2010). Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. *Drug Metabolism & Disposition, 38*(9), 1417–1421.

Killeen, M. J., Linder, M., Pontoniere, P., & Crea, R. (2014). NF-kappabeta signaling and chronic inflammatory diseases: Exploring the potential of natural products to drive new therapeutic opportunities. *Drug Discovery Today, 19*(4), 373–378.

Kirkland, D., Edwards, J., Woehrle, T., & Beilstein, P. (2015). Investigations into the genotoxic potential of olive extracts. *Mutation Research, Genetic Toxicology and Environmental Mutagenesis, 777*, 17–28.

Konstantinidou, V., Khymenets, O., Covas, M. I., de la Torre, R., Muñoz-Aguayo, D., Anglada, R., ... Fito, M. (2009). Time course of changes in the expression of insulin sensitivity-related genes after an acute load of virgin olive oil. *OMICS A Journal of Integrative Biology, 13*(5), 431–438.

Koo, K. A., Kim, S. H., Oh, T. H., & Kim, Y. C. (2006). Acteoside and its aglycones protect primary cultures of rat cortical cells from glutamate-induced excitotoxicity. *Life Sciences, 79*(7), 709–716.

Kotronoulas, A., Pizarro, N., Serra, A., Robledo, P., Joglar, J., Rubio, L., ... De la Torre, R. (2013). Dose-dependent metabolic disposition of hydroxytyrosol and formation of mercapturates in rats. *Pharmacological Research, 77*, 47–56.

Kyriazis, J. D., Aligiannis, N., Polychronopoulos, P., Skaltsounis, A. L., & Dotsika, E. (2013). Leishmanicidal activity assessment of olive tree extracts. *Phytomedicine: International Journal of Phytotherapy and Phytopharmacology, 20*(3–4), 275–281.

Lee, Y. Y., Crauste, C., Wang, H., Leung, H. H., Vercauteren, J., Galano, J. M., ... Lee, J. C. (2016). Extra virgin olive oil reduced polyunsaturated fatty acid and cholesterol oxidation in rodent liver: Is this accounted for hydroxytyrosol-fatty acid conjugation? *Chemical Research in Toxicology, 17*(29), 1689–1698.

Leger, C. L., Carbonneau, M. A., Michel, F., Mas, E., Monnier, L., Cristol, J. P., & Descomps, B. (2005). A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *European Journal of Clinical Nutrition, 59*(5), 727–730.

Li, J., Yang, G., Wang, S., Jiang, L., Liu, X., Geng, C., ... Chen, M. (2012). The protective effects of hydroxytyrosol against ortho-phenylphenol-induced DNA damage in HepG2 cells. *Toxicology Mechanisms and Methods, 22*(6), 432–437.

Liu, Z. H., Fan, W., & Chen, R. C. (2015). 34-Dihydroxyphenylethanol suppresses irradiation-induced pulmonary fibrosis in adult rats. *International Journal of Clinical and Experimental Pathology, 8*(4), 3441–3450.

López de Las Hazas, M. C., Piñol, C., Macià, A., & Motilva, M. J. (2017). Hydroxytyrosol and the colonic metabolites derived from virgin olive oil intake induce cell cycle arrest and apoptosis in colon cancer cells. *Journal of Agricultural and Food Chemistry, 19*. http://dx.doi.org/10.1021/acs.jafc.6b04933 (Epub ahead of print).

Lopez-Huertas, E., & Fonolla, J. (2017). Hydroxytyrosol supplementation increases vitamin C levels in vivo. A human volunteer trial. *Redox Biology, 11*, 384–389.

Lopez-Miranda, J., Perez-Jimenez, F., Ros, E., De Caterina, R., Badimon, L., Covas, M. I., ... Yiannakouris, N. (2010). Olive oil and health: Summary of the II international conference on olive oil and health consensus report Jaen and Cordoba (Spain) 2008. *Nutrition, Metabolism, and Cardiovascular Diseases: NMCD, 20*(4), 284–294.

Luo, C., Li, Y., Wang, H., Cui, Y., Feng, Z., Li, H., Li, Y., ... Liu, J. (2013). Hydroxytyrosol promotes superoxide production and defects in autophagy leading to anti-proliferation and apoptosis on human prostate cancer cells. *Current Cancer Drug Targets, 13*(6), 625–639.

Maalej, A., Bouallagui, Z., Hadrich, F., Isoda, H., & Sayadi, S. (2017). Assessment of *Olea europaea* L. fruit extracts: Phytochemical characterization and anticancer pathway investigation. *Biomedicine & Pharmacotherapy, 90*, 179–186.

Manna, C., Della Ragione, F., Cucciolla, V., Borriello, A., D'Angelo, S., Galletti, P., & Zappia, V. (1999). Biological effects of hydroxytyrosol a polyphenol from olive oil endowed with antioxidant activity. *Advances in Experimental Medicine and Biology, 472*, 115–130.

Martin, M. A., Ramos, S., Granado-Serrano, A. B., Rodriguez-Ramiro, I., Trujillo, M., Bravo, L., & Goya, L. (2010). Hydroxytyrosol induces antioxidant/detoxificant enzymes and Nrf2 translocation via extracellular regulated kinases and phosphatidylinositol-3-kinase/protein kinase B pathways in HepG2 cells. *Molecular Nutrition & Food Research, 54*(7), 956–966.

Martinez-Gonzalez, M. A., Salas-Salvado, J., Estruch, R., Corella, D., Fito, M., & Ros, E. (2015). Benefits of the Mediterranean diet: Insights from the PREDIMED study. *Progress in Cardiovascular Diseases, 58*(1), 50–60.

Mateos, R., Pereira-Caro, G., Saha, S., Cert, R., Redondo-Horcajo, M., Bravo, L., & Kroon, P. A. (2011). Acetylation of hydroxytyrosol enhances its transport across differentiated Caco-2 cell monolayers. *Food Chemistry, 125*(3), 865–872.

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*(14), 4954–4961.

Medina-Martinez, M. S., Truchado, P., Castro-Ibanez, I., & Allende, A. (2016). Antimicrobial activity of hydroxytyrosol: A current controversy. *Bioscience Biotechnology and Biochemistry, 80*(4), 801–810.

Menendez, J. A., & Lupu, R. (2006). Oncogenic properties of the endogenous fatty acid metabolism: Molecular pathology of fatty acid synthase in cancer cells. *Current Opinion in Clinical Nutrition and Metabolic Care, 9*(4), 346–357.

Menendez, J. A., Vazquez-Martin, A., Colomer, R., Brunet, J., Carrasco-Pancorbo, A., Garcia-Villalba, R., ... Segura-Carretero, A. (2007). Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin) in HER2-overexpressing breast cancer cells. *BMC Cancer, 7*, 80.

Minuti, L., Pellegrino, R. M., & Tesei, I. (2006). Simple extraction method and gas chromatography-mass spectrometry in the selective ion monitoring mode for the determination of phenols in wine. *Journal of Chromatography, A, 1114*(2), 263–268.

Miro-Casas, E., Covas, M. I., Farre, M., Fito, M., Ortuno, J., Weinbrenner, T., ... De la Torre, R. (2003). Hydroxytyrosol disposition in humans. *Clinical Chemistry, 49*, 945–952.

Montedoro, G., Servili, M., Baldioli, M., & Miniati, E. (1992). Simple and hydrolyzable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. *Journal of Agricultural and Food Chemistry, 40*, 1571–1576.

Mosele, J. I., Martin-Pelaez, S., Macia, A., Farras, M., Valls, R. M., Catalan, U., & Motilva, M. J. (2014). Faecal microbial metabolism of olive oil phenolic compounds: In vitro and in vivo approaches. *Molecular Nutrition & Food Research, 58*(9), 1809–1819.

Mullen, G. E., & Yet, L. (2015). Progress in the development of fatty acid synthase inhibitors as anticancer targets. *Bioorganic & Medicinal Chemistry Letters, 25*(20), 4363–4369.

Muñoz-Marin, J., De La Cruz, J. P., Guerrero, A., Lopez-Leiva, I., Lopez-Villodres, J. A., Reyes, J. J., ... Gonzalez-Correa, J. A. (2012). Cytoprotective effect of hydroxytyrosyl alkyl ether derivatives after oral administration to rats in a model of glucose-oxygen deprivation in brain slices. *Journal of Agricultural and Food Chemistry, 60*(31), 7659–7664.

Nogueira, V., Park, Y., Chen, C. C., Xu, P. Z., Chen, M. L., Tonic, I., ... Hay, H. (2008). Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell, 14*(6), 458–470.

Notarnicola, M., Pisanti, S., Tutino, V., Bocale, D., Rotelli, M. T., Gentile, A., ... Caruso, M. G. (2011). Effects of olive oil polyphenols on fatty acid synthase gene expression and activity in human colorectal cancer cells. *Genes & Nutrition, 6*(1), 63–69.

Ocakoglu, D., Tokatli, F., Ozen, B., & Korel, F. (2009). Distribution of simple phenols, phenolic acids and flavonoids in Turkish monovarietal extra virgin olive oils for two harvest years. *Food Chemistry, 113*(2), 401–410.

O'Dowd, Y., Driss, F., Dang, P. M., Elbim, C., Gougerot-Pocidalo, M. A., Pasquier, C., & El-Benna, J. (2004). Antioxidant effect of hydroxytyrosol a polyphenol from olive oil: Scavenging of hydrogen peroxide but not superoxide anion produced by human neutrophils. *Biochemical Pharmacology, 68*(10), 2003–2008.

Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., Würtele, G., Spiegelhalder, B., & Bartsch, H. (2000). Olive-oil consumption and health: The possible role of antioxidants. *The Lancet Oncology, 1*(2), 107–112.

Park, G., Kim, H. G., Hong, S. P., Kim, S. Y., & Oh, M. S. (2014). Walnuts (seeds of *Juglandis sinensis* L,) protect human epidermal keratinocytes against UVB-induced mitochondria-mediated apoptosis through upregulation of ROS elimination pathways. *Skin Pharmacology and Physiology, 27*(3), 132–140.

Pastor, A., Rodriguez-Morato, J., Olesti, E., Pujadas, M., Perez-Mana, C., Khymenets, O., ... De la Torre, R. (2016). Analysis of free hydroxytyrosol in human plasma following the administration of olive oil. *Journal of Chromatography A, 1437*, 183–190.

Perez-Bonilla, M., Salido, S., van Beek, T. A., & Altarejos, J. (2014). Radical-scavenging compounds from olive tree (*Olea europaea* L,) wood. *Journal of Agricultural and Food Chemistry, 62*(1), 144–151.

Perez-Mana, C., Farre, M., Pujadas, M., Mustata, C., Menoyo, E., Pastor, A., ... De La Torre, R. (2015). Ethanol induces hydroxytyrosol formation in humans. *Pharmacological Research, 95-96*, 27–33.

Poudyal, H., Campbell, F., & Brown, L. (2010). Olive leaf extract attenuates cardiac hepatic and metabolic changes in high carbohydrate- high fat-fed rats. *The Journal of Nutrition, 140*(5), 946–953.

Procopio, A., Alcaro, S., Nardi, M., Oliverio, M., Ortuso, F., Sacchetta, P., et al. (2009). Synthesis biological evaluation and molecular modeling of oleuropein and its semisynthetic derivatives as cyclooxygenase inhibitors. *Journal of Agricultural and Food Chemistry, 57*(23), 11161–11167.

Quirantes-Pine, R., Zurek, G., Barrajon-Catalan, E., BaSsmann, C., Micol, V., Segura-Carretero, A., & Fernandez-Gutierrez, A. (2013). A metabolite-profiling approach to assess the uptake and metabolism of phenolic compounds from olive leaves in SKBR3 cells by HPLC-ESI-QTOF-MS. *Journal of Pharmaceutical and Biomedical Analysis, 72*, 121–126.

Rafehi, H., Smith, A. J., Balcerczyk, A., Ziemann, M., Ooi, J., Loveridge, S. J., ... Karagiannis, T. C. (2012). Investigation into the biological properties of the olive polyphenol hydroxytyrosol: Mechanistic insights by genome-wide mRNA-Seq analysis. *Genes & Nutrition, 7*(2), 343–355.

Ramirez-Tortosa, M. C., Pulido-Moran, M., Granados, S., Gaforio, J. J., & Quiles, J. L. (2014). Hydroxytyrosol as a component of the Mediterranean diet and its role in disease prevention. In V. R. Preedy, & R. R. Watson (Vol. Eds.), *The Mediterranean Diet: An Evidence-Based Approach. vol. 1. The Mediterranean Diet: An Evidence-Based Approach* (pp. 205–215). Elsevier Science.

Rashid, A., Pizer, E. S., Moga, M., Milgraum, L. Z., Zahurak, M., Pasternack, G. R., ... Hamilton, S. R. (1997). Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. *The American Journal of Pathology, 150*(1), 201–208.

Richard, N., Arnold, S., Hoeller, U., Kilpert, C., Wertz, K., & Schwager, J. (2011). Hydroxytyrosol is the major anti-inflammatory compound in aqueous olive extracts and impairs cytokine and chemokine production in macrophages. *Planta Medica, 77*(17), 1890–1897.

Rodríguez-Morató, J., Boronat, A., Kotronoulas, A., Pujadas, A., Pastor, A., & Olesti, E. (2016). Metabolic disposition and biological significance of simple phenols of dietary origin: Hydroxytyrosol and tyrosol. *Drug Metabolism Reviews, 48*, 218–236.

Rodriguez-Morato, J., Xicota, L., Fito, M., Farre, M., Dierssen, M., & de la Torre, R. (2015). Potential role of olive oil phenolic compounds in the prevention of neurodegenerative diseases. *Molecules (Basel Switzerland), 20*(3), 4655–4680.

Romero, C., Brenes, M., Yousfi, K., Garcia, P., Garcia, A., & Garrido, A. (2004). Effect of cultivar and processing method on the contents of polyphenols in table olives. *Journal of Agricultural and Food Chemistry, 52*(3), 479–484.

Roowi, S., Mullen, W., Edwards, C. A., & Crozier, A. (2009). Yoghurt impacts on the excretion of phenolic acids derived from colonic breakdown of orange juice flavanones in humans. *Molecular Nutrition & Food Research, 53*, S68–75.

Rosignoli, P., Fuccelli, R., Sepporta, M. V., & Fabiani, R. (2016). In vitro chemo-preventive activities of hydroxytyrosol: the main phenolic compound present in extra-virgin olive oil. *Food & Function, 7*, 301–307.

Rubió, L., Farras, M., de la Torre, R., Macià, A., Romero, M. P., Valls, R. M., ... Motilva, M. J. (2014). Metabolite profiling of olive oil and thyme phenols after a sustained intake of two phenol-enriched olive oils by humans: Identification of compliance markers. *Food Research International, 65*, 59–68.

Rubio, L., Valls, R. M., Macia, A., Pedret, A., Giralt, M., Romero, M. P., ... Motilva, M. J. (2012). Impact of olive oil phenolic concentration on human plasmatic phenolic metabolites. *Food Chemistry, 135*(4), 2922–2929.

Salas-Salvado, J., Bullo, M., Estruch, R., Ros, E., Covas, M. I., Ibarrola-Jurado, N., ... Martinez-gonzalez, M. A. (2014). Prevention of diabetes with Mediterranean diets: A subgroup analysis of a randomized trial. *Annals of Internal Medicine, 160*(1), 1–10.

Salucci, S., Burattini, S., Curzi, D., Buontempo, F., Martelli, A. M., Zappia, G., ... Battistelli, M. (2014). Antioxidants in the prevention of UVB-induced keratynocyte apoptosis. *Journal of Photochemistry and Photobiology B: Biology, 141*, 1–9.

Sarsour, E. H., Kumar, M. G., Kalen, A. L., Goswami, M., Buettner, G. R., & Goswami, P. C. (2012). MnSOD activity regulates hydroxytyrosol-induced extension of chronological lifespan. *Age (Dordrecht, Netherlands), 34*(1), 95–109.

Schaffer, S., Muller, W. E., & Eckert, G. P. (2010). Cytoprotective effects of olive mill wastewater extract and its main constituent hydroxytyrosol in PC12 cells. *Pharmacological Research, 62*(4), 322–327.

Schaffer, S., Podstawa, M., Visioli, F., Bogani, P., Muller, W. E., & Eckert, G. P. (2007). Hydroxytyrosol-rich olive mill wastewater extract protects brain cells in vitro and ex vivo. *Journal of Agricultural and Food Chemistry, 55*(13), 5043–5049.

Sclip, A., Antoniou, X., Colombo, A., Camici, G. G., Pozzi, L., Cardinetti, D., ... Borsello, T. (2011). C-Jun N-terminal kinase regulates soluble Ab oligomers and cognitive impairment in AD mouse model. *The Journal of Biological Chemistry, 286*, 43871–43880.

Scoditti, E., Nestola, A., Massaro, M., Calabriso, N., Storelli, C., De Caterina, R., & Carluccio, M. A. (2014). Hydroxytyrosol suppresses MMP-9 and COX-2 activity and expression in activated human monocytes via PKCalpha and PKCbeta1 inhibition. *Atherosclerosis, 232*(1), 17–24.

Serra, A., Rubio, L., Borras, X., Macia, A., Romero, M. P., & Motilva, M. J. (2012). Distribution of olive oil phenolic compounds in rat tissues after administration of a phenolic extract from olive cake. *Molecular Nutrition & Food Research, 56*(3), 486–496.

Servili, M., Selvaggini, R., Esposto, S., Taticchi, A., Montedoro, G. F., & Morozzi, G. (2004). Health and sensory properties of virgin olive oil hydrophilic phenols: Agronomic and technological aspects of production that affect their occurrence in the oil. *Journal of Chromatography A, 1054*, 113–127.

Soni, M. G., Burdock, G. A., Christian, M. S., Bitler, C. M., & Crea, R. (2006). Safety assessment of aqueous olive pulp extract as an antioxidant or antimicrobial agent in foods. *Food and Chemical Toxicology: An International Journal Published for the British Industrial Biological Research Association, 44*(7), 903–915.

Suarez, M., Valls, R. M., Romero, M. P., Macia, A., Fernandez, S., Giralt, M., ... Motilva, M. J. (2011). Bioavailability of phenols from a phenol-enriched olive oil. *The British Journal of Nutrition, 106*(11), 1691–1701.

Sun, L., Luo, C., & Liu, J. (2014). Hydroxytyrosol induces apoptosis in human colon cancer cells through ROS generation. *Food & Function, 5*(8), 1909–1914.

Tasset, I., Pontes, A. J., Hinojosa, A. J., de la Torre, R., & Tunez, I. (2011). Olive oil reduces oxidative damage in a 3-nitropropionic acid-induced Huntington's disease-like rat model. *Nutritional Neuroscience, 14*(3), 106–111.

Terzuoli, E., Giachetti, A., Ziche, M., & Donnini, S. (2016). Hydroxytyrosol, a product from olive oil, reduces colon cancer growth by enhancing epidermal growth factor receptor degradation. *Molecular Nutrition & Food Research, 60*, 519–529.

Toteda, G., Lupinacci, S., Vizza, D., Bonofiglio, R., Perri, E., Bonofiglio, M., ... Perri, A. (2017). High doses of hydroxytyrosol induce apoptosis in papillary and follicular thyroid cancer cells. *Journal of Endocrinological Investigation, 40*(2), 153–162.

Trujillo, M., Gallardo, E., Madrona, A., Bravo, L., Sarria, B., Gonzalez-Correa, J. A., ... Espartero, J. L. (2014). Synthesis and antioxidant activity of nitrohydroxytyrosol and its acyl derivatives. *Journal of Agricultural and Food Chemistry, 62*(42), 10297–10303.

Tuck, K. L., Freeman, M. P., Hayball, P. J., Stretch, G. L., & 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. *The Journal of Nutrition, 131*(7), 1993–1996.

Tutino, V., Caruso, M. G., Messa, C., Perri, E., & Notarnicola, M. (2012). Antiproliferative antioxidant and anti-inflammatory effects of hydroxytyrosol on human hepatoma HepG2 and Hep3B cell lines. *Anticancer Research, 32*(12), 5371–5377.

Varela-Lopez, A., Bullon, P., Battino, M., Ramirez-Tortosa, M., Ochoa, J. J., Cordero, M. D., et al. (2015). Coenzyme Q protects against age-related alveolar bone loss associated to n-6 polyunsaturated fatty acid rich-diets by modulating mitochondrial mechanisms. *The Journals of Gerontology, Series A Biological Sciences and Medical Sciences, 71*(5), 593–600.

Vilaplana-Perez, C., Aunon, D., Garcia-Flores, L. A., & Gil-Izquierdo, A. (2014). Hydroxytyrosol and potential uses in cardiovascular diseases cancer and AIDS. *Frontiers in Nutrition, 1*, 18.

Visioli, F. (2015). Xenobiotics and human health: A new view of their pharma-nutritional

role. *PharmaNutrition, 3*, 60–64.
Visioli, F., Bellomo, G., & Galli, C. (1998). Free radical-scavenging properties of olive oil polyphenols. *Biochemical and Biophysical Research Communications, 247*(1), 60–64.
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. *The Journal of Nutrition, 133*(8), 2612–2615.
Vissers, M. N., Zock, P. L., Roodenburg, A. J. C., Leenen, R., & Katan, M. B. (2002). Olive oil phenols are absorbed in humans. *The Journal of Nutrition, 132*(3), 409–417.
Wang, Y., Kuhajda, F. P., Sokoll, L. J., & Chan, D. W. (2001). Two-site ELISA for the quantitative determination of fatty acid synthase. *Clinica Chimica Acta, 304*(1–2), 107–115.
Warleta, F., Quesada, C. S., Campos, M., Allouche, Y., Beltran, G., & Gaforio, J. J. (2011). Hydroxytyrosol protects against oxidative DNA damage in human breast cells. *Nutrients, 3*(10), 839–857.
Zhang, X., Cao, J., & Zhong, L. (2009). Hydroxytyrosol inhibits pro-inflammatory cytokines iNOS and COX-2 expression in human monocytic cells. *Naunyn Schmiedebergs Archives of Pharmacology, 379*(6), 581–586.
Zhang, X., Jiang, L., Geng, C., Yoshimura, H., & Zhong, L. (2008). Inhibition of acrylamide genotoxicity in human liver-derived HepG2 cells by the antioxidant hydroxytyrosol. *Chemico-Biological Interactions, 176*(2–3), 173–178.
Zheng, A., Li, H., Cao, K., Xu, J., Zou, X., Li, Y., ... Feng, Z. (2015). Maternal hydroxytyrosol administration improves neurogenesis and cognitive function in prenatally stressed offspring. *The Journal of Nutritional Biochemistry, 26*(2), 190–199.
Zheng, A., Li, H., Xu, J., Cao, K., Li, H., Pu, W., ... Feng, Z. (2015). Hydroxytyrosol improves mitochondrial function and reduces oxidative stress in the brain of db/db mice: Role of AMP-activated protein kinase activation. *The British Journal of Nutrition, 113*(11), 1667–1676.
Zhu, L., Liu, Z., Feng, Z., Hao, J., Shen, W., Li, X., ... Liu, J. (2010). Hydroxytyrosol protects against oxidative damage by simultaneous activation of mitochondrial biogenesis and phase II detoxifying enzyme systems in retinal pigment epithelial cells. *The Journal of Nutritional Biochemistry, 21*(11), 1089–1098.
Zoric, N., Horvat, I., Kopjar, N., Vucemilovic, A., Kremer, D., Tomic, S., & Kosalec, I. (2013). Hydroxytyrosol expresses antifungal activity in vitro. *Current Drug Targets, 14*(9), 992–998.
Zou, X., Feng, Z., Li, Y., Wang, Y., Wertz, K., Weber, P., ... Liu, J. (2012). Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: Activation of Nrf2 and JNK-p62/SQSTM1 pathways. *The Journal of Nutritional Biochemistry, 23*(8), 994–1006.
Zrelli, H., Matsuoka, M., Kitazaki, S., Araki, M., Kusunoki, M., Zarrouk, M., & Miyazaki, H. (2011). Hydroxytyrosol induces proliferation and cytoprotection against oxidative injury in vascular endothelial cells: Role of Nrf2 activation and HO-1 induction. *Journal of Agricultural and Food Chemistry, 59*(9), 4473–4482.
Zwane, R. E., Parker, A., Kudanga, T., Davids, L. M., & Burton, S. G. (2012). Novel biocatalytically produced hydroxytyrosol dimer protects against ultraviolet-induced cell death in human immortalized keratinocytes. *Journal of Agricultural and Food Chemistry, 60*(46), 11509–11517.

学霸图书馆
www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页　　文献云下载　　图书馆入口　　外文数据库大全　　疑难文献辅助工具