Review

Bioactive compounds in wine: Resveratrol, hydroxytyrosol and melatonin: A review

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Abstract

Regular moderate wine consumption is often associated with reduced morbidity and mortality from a variety of chronic diseases in which inflammation is the root cause. This review is focused on three of the numerous bioactive compounds present in wine: resveratrol, hydroxytyrosol and melatonin. Resveratrol and hydroxytyrosol are polyphenols. Melatonin, recently described in wine, is an indoleamine. Their structures, concentrations in wine, bioavailability, pharmacokinetic and health promoting properties are reviewed. Resveratrol seems to be one of the most promising compounds due to its bioactivity, with wine being the main source of resveratrol in diet. Hydroxytyrosol, which its main source in diet is olive oil has been also found in both red and white wine in considerable amounts. Melatonin has been found in wine in low amounts. However, both high bioactivity and bioavailability have been attributed to it. They show antioxidant, cardioprotective, anticancer, antidiabetic, neuroprotective and antiaging activities. However, human studies are still in the initial stages and therefore further studies are needed.

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1. Introduction

The relationship between diet and health has developed an intense research in bioactive compounds in foods. Wine seems to be an essential component and may be partially responsible for health-promoting properties observed among the Mediterranean population. The starting point for wine and health studies was the “French Paradox”. Renaud and De Lorgeril (1992) published a study confirming the association between death by cardiovascular disease and dietary intake. Despite a diet being traditionally high in saturated fats, myocardial infarction rates in France are 40% lower than in the rest of Europe. If wine intake was considered, the French population perfectly fitted the regression model.

From all the studies that have been carried out in the health-and-wine field, it can be affirmed that supplementing the regular diet with red wine increases the total antioxidant capacity in plasma, HDL lipoprotein, fibrinolytic and antithrombin activity. Moreover, it reduces oxidative damage and platelet aggregation. Studies from different parts of the world with diverse population groups, suggested that moderate consumption (1–2 glasses per day) of wine drinks reduce cardiovascular risk (Avellone et al., 2006; Bertelli & Das, 2009; Leighton et al., 1999; Mezzano et al., 2001; Rimm et al., 1995).

Although less evident, wine could have an influence on cancer risk. Moderate consumption of wine reduces the risk of non-Hodgkin’s lymphoma (Briggs et al., 2002), adenocarcinoma of the oesophagus, prostate cancer (Platz, Leitzmann, Rimm, & Willett, 2004; Schoonen, Salinas, Kiemeneij, & Stanford, 2005; Schurman, Goldbohm, & Van den Brandt, 1999) and gastric cardia (Gammon et al., 1997). However, other authors have not found any relationship (Bessaoud & Daures, 2008; Sutcliffe et al., 2007) and some of them even found a negative effect (Longnecker, Orza, Adams, Vioque, & Chalmers, 1990).

Among wines, red wine is considered to have a more protective effect due to its greater content in antioxidant substances released from the grape’s skin and seeds (mainly polyphenols). A bottle of red wine contains a total of 1.8 g/l of polyphenols, whereas a bottle of white wine contains only 0.2–0.3 g/l of polyphenols (Bertelli & Das, 2009). In the making of white wine, skin and seeds are removed immediately from the must, which is left to ferment without them. As in vitro antioxidant capacity is strongly correlated with total polyphenol content in vitro, white wines present from five to ten times lesser antioxidant activity than red wines (Lugasi & Hovari, 2003). However, wine additionally contains a high amount of hydroxycinnamic acids, tyrosol and hydroxytyrosol, which are also known to have some antioxidant properties.

The findings that red wine presented more health-promotion activity than beer or spirits caused research attention to focus on polyphenic compounds. Several studies have been undertaken to differentiate the effects of phenolic and other non-alcohol components of wine from those due to alcohol. In animal models it has been demonstrated that a red wine polyphenolic extract prevents the development of cardiovascular problems and cancer. Al-Awwadi et al. (2004) compared blood pressure, heart weight and reactive oxygen species in rats whose feed had been supplemented with the polyphenolic extract, ethanol or both polyphenolic extract and ethanol together. They concluded that the polyphenolic extract was the most effective supplement for reducing cardiovascular risk. Clifford et al. (1996) demonstrated that the consumption of de-alcoholized red wine as a part of a defined complete diet delayed tumor onset in transgenic mice.

All these effects could be understood due to the synergistic effects that may occur among bioactive compounds. Synergy has been reported among the three phenols, resveratrol, caffeic acid and catechin (Norata et al., 2007; Pignatelli, Cao, & Zhu, 2006). Despite their relatively low plasma concentrations following moderate wine consumption, this synergy gives them useful biological activity, such as the inhibition of oxidative stress. Interaction between polyphenols may influence their kinetics and metabolism.

In this review we focus on three bioactive compounds present in wine resveratrol, hydroxytyrosol and melatonin. Resveratrol is one of the most promising compounds due to its bioactivity, with wine being the main source of resveratrol in diet. Hydroxytyrosol is a potent antioxidant mainly found in olive oil, but wine has been described as an additional source of hydroxytyrosol in the diet. Melatonin has been recently found in wine at low concentrations. However, its high bioactivity justifies its inclusion in the present review.

2. Resveratrol

2.1. Structure and concentration in wine

Resveratrol (3,5,4’-trans-trihydroxystilbene, Fig. 1a) is a member of the stilbene family of phenolic compounds. Langcake and Pryce (1976) detected it in Vitis vinifera grapevines. Resveratrol is synthesized by leaf tissues in response to fungal infection or exposure to ultraviolet light but, until 1992, it was not detected in wine (Siemann & Creasy, 1992).

Resveratrol and stilbenes in general are commonly found in many plants. However, their dietary sources are rather limited: peanut and its derivatives, pistachio, berries, dark chocolate, and grapes as well as their derivatives. Of all of them, grapes present...
The amount of resveratrol in wine varies widely depending on many factors: grape variety, geographic region, agronomic factors, climatic factors, plant stress conditions and oenological practices. Regarding optimum oenological practices, all the processes that affect resveratrol biosynthesis. Concentrations ranging from undetectable to 14.3 mg/l have been described (Frémont, 2002; Souto et al., 2001; Melzoch, Hanzlíková, Filip, Buckiová, and Smidrkal (2001); Mark, Nikfardjam, Avar, and Ohmacht (2005)).

Pathogenic attack (Roldán, Palacios, Caro, & Pérez, 2003; González-Candelas, Gil, Lamuela-Raventós, & Ramón, 2003; Romanazzi, Gabler, & Smilanick, 2006) and UVC (Becker et al., 2003; González-Candelas, Gil, Lamuela-Raventós, & Ramón, 2000) are potent factors that make resveratrol content in grapes increase and consequently in wines too. Another strategy proposed as responsible for increasing resveratrol in wine is the use of transgenic yeast (Becker et al., 2003; González-Candelas, Gil, Lamuela-Raventós, & Ramón, 2000). These treatments are now applied specifically with the aim of producing wines enriched in resveratrol and, what is commercially even more important, wines with a constant and predictable high level of resveratrol over the years or vintages. This would represent a proven added value for the product in nutritional and health terms, which can be exploited in the market (Barreiro-Hurlé, Colombo, & Cantos-Villar, 2008). In fact, commercial application has already been found (http://www.drnorrie.info/html/rew.html).

Table 1

<table>
<thead>
<tr>
<th>Variety region</th>
<th>trans-Resveratrol (mg/l)</th>
<th>Samples average</th>
<th>Sample number</th>
<th>References</th>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>Nq</td>
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<td>1</td>
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<tr>
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<td>5.1</td>
<td>4.0 ± 1.0</td>
<td>3</td>
</tr>
<tr>
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<td>Nq</td>
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<td>1</td>
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<tr>
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</tr>
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<tr>
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<td>Nq</td>
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<td>1</td>
</tr>
<tr>
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<tr>
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<tr>
<td>Japan</td>
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<td>Nq</td>
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<td>1</td>
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<tr>
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<tr>
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</tr>
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<td>1.7 ± 1.7</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Spain</td>
<td>0.2</td>
<td>2.5</td>
<td>1.3 ± 0.7</td>
<td>12</td>
</tr>
</tbody>
</table>

Nq, no quantified.
**Fig. 2.** Biosynthesis pathway of resveratrol (Ferrer, Austin, Steward, & Noel, 2008).

(Poutraud et al., 2007); of course, many of these compounds, including resveratrol, piceid, astringin and stilbene oligomers (viniferins), are also found in finished wines (Buiarelli, Coccoli, Jasionowska, Merolle, & Terracciano, 2007; Guebalia et al., 2006; Ribeiro de Lima et al., 1999; Vitrac et al., 2005). However, the concentration of these compounds in wine is low and therefore, their bioactivity has been investigated less than that of resveratrol.

Two different types of reaction lead to the formation of stilbene derivatives from trans-resveratrol (the initial compound in the stilbene pathway) in susceptible grapevines and resistant cultivars, respectively. In susceptible grapevines resveratrol is synthesized in large amounts early after an infection, but it is rapidly glycosylated into a non-toxic compound: piceid. In resistant varieties resveratrol is synthesized in large amounts early after an infection, but it is rapidly glycosylated into toxic viniferins (Pezet, Gindre, Viret, & Spring, 2004).

### 2.2. Bioavailability and pharmacokinetics

Numerous studies in animals and humans have shown that the bioavailability of unconjugated resveratrol is low. At least 70% of resveratrol ingested is absorbed, and readily metabolized to form mainly glucuronide and sulphate derivatives. The colon micro-flora can produce the metabolite dihydroresveratrol. Resveratrol metabolites reach their maximum concentration in plasma approximately 30 min after intake; the half-life of total metabolites is approximately 9.2 h (Walle, Hsieh, DeLegge, Oatis, & Walle, 2004). Plasma concentration of resveratrol and its metabolites decrease approximately 30 min after intake; the half-life of total metabolites is approximately 24 h (Marier et al., 2002). Five distinct metabolites are present in the urine after moderate consumption of red wine: resveratrol monosulphate, two isomeric forms of resveratrol monoglucuronide, dihydroresveratrol monosulphate and dihydroresveratrol (Vitaiglione et al., 2005). In low density lipoprotein samples from volunteers who had ingested 250 ml of red wine containing a known quantity of resveratrol, up to six metabolites have been measured: trans-resveratrol-3-O-glucuronide, cis-resveratrol-3-O-glucuronide, free trans-resveratrol and, tentatively, resveratrol-4′-O-glucuronide and trans-resveratrol-4-O-glucoside (Urpi-Sarda et al., 2005).

Since the in vivo concentration of individual metabolites from ingested resveratrol can be much higher than that of resveratrol itself, further studies of the activity of its metabolites are needed.

Bertelli, Giovannini, Stradi, Bertelli, and Tillement (1996) demonstrated that resveratrol present in red wine (6.5 mg/l as cis and trans forms) reached target tissues in rats. Plasma, urine and tissue levels of trans- and cis-resveratrol were measured in rats after the administration of a single dose of 4 ml of red wine containing 6.5 mg/l total resveratrol and after the administration of 2 ml red wine containing the same amount of the stilbene for 15 days. Resveratrol was found not only in the plasma and urine, but also in the heart, liver and kidneys. Resveratrol plasma levels within the same range were subsequently found also in a clinical trial in 10 healthy volunteers who drank 300 ml of red wine for 15 days, but not in another 10 healthy volunteers who drank 300 ml of white wine for 15 days. In a randomized, single-blind, crossover trial, in which 13 healthy volunteers drank wine, ethanol and water on three separate occasions, 2 weeks apart, the mean ± SE plasma resveratrol plasma level was 24.8 ± 5.8 μg/l after the ingestion of 155 ml of Australian Pinot Noir wine and 43.0 ± 9.4 μg/l after the ingestion of 310 ml. It is important to bear in mind that three servings (approx 450 ml) are more than sufficient to achieve plasma levels of free trans-resveratrol within the range of 100 nM–1 μM (Bertelli & Das, 2009).

Resveratrol proved in vitro anticarcinogenic activity at doses ranging from 5 to 100 mM, meanwhile the doses for the prevention of cardiovascular disease are between 100 nM and 1 mM (Bertelli, 2007), which means that at modest dosages, resveratrol was pharmacologically active both in vitro and in vivo. These authors suggested that an average drinker of wine could, particularly in the long term, absorb a sufficient quantity of resveratrol to explain the beneficial effect of red wine on human health. More importantly, this could help to explain how a relatively low dose of resveratrol obtained from red wine or other dietary sources could be therapeutic in some cases (Bertelli, Bertelli, Goozini, & Giovannini, 1998). A dose-dependent response has been observed, and, at higher but pharmacologically achievable doses, protective effects of resveratrol are more frequently observed, and the results are more dramatic (Chen, Tseng, Lai, & Chen, 2004).

Resveratrol binds to albumin. It has been suggested that albumin could be a natural polyphenol reservoir in the in vivo context, where it might play a pivotal role in the distribution and bioavailability of circulating resveratrol (Jannin et al., 2004). The accumulation of resveratrol in other organs such as the heart, liver and lungs after chronic administration was described for the first time in 1996 (Bertelli et al., 1996) and has more recently been confirmed (El–Mohsen et al., 2006; Vitrac et al., 2003) and extended to the bile, stomach and kidneys (De Santi, Pietrabissa, Spisi, Mosca, & Pacifici, 2000).

It is also worth considering the potential interactions of resveratrol with other constituents of the diet. Resveratrol has been shown to synergize with both quercetin and ellagic acid in the induction of apoptosis in human leukemia cells (Mertens–Talcott & Percival, 2005), with ethanol in the inhibition of iNOS expression (Chen, Mattiacci, Hwang, Shah, & Fong, 2000), with vitamin E in the prevention of lipid peroxidation (Fang et al., 2002), with catechin in the protection of PC12 cells from β-amyloid toxicity (Conte, Pellegrini, & Tagliazucchi, 2003), with nucleoside analogues in the inhibition of HIV1 replication in cultured T lymphocytes (Heredia, Davis, & Redfield, 2000), and with tyrosol and β-sitosterol in modulation of LDL oxidative stress and PGE2 synthesis (Vivancos & Moreno, 2008). The absorptive efficiency of trans-resveratrol, (+)-catechin and quercetin was investigated after oral application to...
healthy human subjects in three media (white wine, grape juice and vegetable homogenate). The absorption of these three polyphenols was equivalent in the different matrices but, at peak concentrations of 10–40 nmol/l, it was inadequate to permit circulating concentrations of 5–100 nmol/l consistent with in vitro biological activity (Goldberg, Yan, & Soleas, 2003). Moreover, one finding that has often been overlooked is that quercetin, which is also present in red wine, is a picomolar inhibitor of resveratrol sulphonylation in both the liver and duodenum, thus increasing the bioavailability of unconjugated resveratrol (De Santi et al., 2000). As regards toxicity effects of resveratrol, it has been established than all these health benefits are not coupled with adverse side effects, unless extremely high doses are administered. Juan, Vinardell, and Planas (2002) found no adverse effects in rats at consumption for 28 days of the quantity of resveratrol equivalent to 1000-times the content of this compound in red wine. Recently similar results were found. A 28-day study was performed on rats, where Resvida™ (high purity resveratrol content) caused no adverse effects in rats at 50, 150 and 500 mg/kg body weight/day. Similarly, in a 90-day study, Resvida™ did not cause any adverse effects in rats at up to 700 mg/kg body weight/day, the highest dose tested (Williams, Burdock, Edwards, Beck, & Bausch, 2009).

2.3. Health-promoting properties

2.3.1. Antioxidant activity

Normal cellular metabolism generates reactive oxygen intermediates (ROI) such as superoxide, hydrogen peroxide and hydroxyl radicals, which are usually detoxified by intracellular enzymes such as glutathione, superoxide dismutase and catalase. However, an abnormal accumulation of ROIs can happen, which is commonly referred to as “oxidative stress”. Exposure of macromolecules (lipids, protein, DNA) to ROIs results in their oxidative modifications with deleterious potential (Arthur, Niu, Rigby, Steer, & Jeffrey, 2008).

Resveratrol has an intrinsic antioxidant capacity that could be related to its chemopreventive effects. In vitro, the induction of detoxification enzymes has been shown after low doses of resveratrol (Li, Cao, & Zhu, 2006). In vivo, resveratrol has been shown to increase plasma antioxidant capacity and to decrease lipid peroxidation (Wenzel, Soldo, Erbersdobler, & Somoza, 2005; Whitehead, Robinson, Allaway, Syms, & Hale, 1995), which is strongly associated with the risk of coronary heart disease and myocardial infarction (Holvoet, 2004). Studies in rats, pigs and humans seem to indicate that resveratrol can suppress pathological increases in the peroxidation of lipids and other macromolecules in vivo, but whether the mechanism is direct, indirect or both, is not clear yet (Baur et al., 2006).

2.3.2. Cardioprotective capacity

Resveratrol protects the cardiovascular system in a multidimensional way (Hao & He, 2004). The most important point is that resveratrol, when at very low concentration, inhibits apoptotic cell death, thereby providing protection from various diseases including myocardial ischemic reperfusion injury, atherosclerosis and ventricular arrhythmias. In higher doses it facilitates apoptotic cell death and behaves as a chemo-preventive alternative (Das & Das, 2007).

Resveratrol modulates lipid and lipoprotein metabolism; it may suppress pathological increases of peroxidation in macromolecules such as lipids. In 1982 it was shown that resveratrol inhibits the deposition of cholesterol and triglycerides in the liver of rats, and decreases the rate of hepatic triglyceride synthesis (Arichi et al., 1982). Later, it was demonstrated that trans-resveratrol inhibits LDL peroxidation in vitro more than an extract of red wine (Frankel, Kanner, German, Parks, & Kinsella, 1993). Resveratrol has been detected in LDL particles from humans after consumption of red wine (Urpi-Sarda et al., 2005).

Platelet aggregation is one of the major contributors to the process of atherosclerosis. Resveratrol prevents platelet aggregation in vitro (Bertelli et al., 1995) and in vivo (Wang et al., 2002). Further research has shown that resveratrol reduces the formation of atherosclerotic plaques and restores flow-mediated dilation in rabbits fed a high-cholesterol diet (Wang et al., 2005).

Resveratrol also promotes vasodilatation through multiple mechanisms, mainly the stimulation of Ca²⁺-activated K⁺ channels and the enhancement of nitric oxide signalling in the endothelium, and therefore it can exert vaso-relaxant activity (Li, Chen, & Wu, 2000; Orallo et al., 2002). In guinea pigs, the addition of resveratrol to drinking water for 16 days (~14 mg per kg body weight) significantly increased its capacity to eliminate oxidants in cardiac muscle (Floreni, Napoli, Quintieri, & Palatini, 2003). The main mechanism seems to be an increase in nitric oxide concentrations by both increasing the expression of nitric oxide synthase and decreasing the inactivation of nitric oxide by free radicals.

2.3.3. Anticancer activity

Jang et al. (1997) reported the ability of resveratrol to inhibit carcinogenesis at multiple stages (initiation, promotion and progression). Their finding that topical application of resveratrol reduced the number of skin tumours per mouse by up to 98% triggered research on resveratrol all around the world. Resveratrol could slow tumour development through multiple complementary mechanisms. It inhibits the enzymatic activity of both forms of cyclooxygenase, which implies a risk reduction of developing many cancers. Another mechanism by which resveratrol could combat tumour formation is induction of cell cycle arrest and apoptosis. The anti-proliferative and pro-apoptotic effects of resveratrol in tumour cell lines have been extensively documented in vitro (Aggarwal et al., 2004). This is supported by down regulation of cell cycle proteins (Schneider et al., 2001) and increases in apoptosis (Garvin, Ollinger, & Dabrosin, 2006) in tumour models in vivo. However, in some in vivo experiments resveratrol failed to affect cancer, which suggests that other factors such as dosage, delivery method, tumour origin and other components of the diet could all contribute to the efficacy of resveratrol treatment. Overall, in vivo studies clearly show great promise for this molecule in the treatment of cancers, although studies of the association between red wine consumption and cancer in humans are still in their initial stages.

The efficacy of resveratrol in colorectal cancer has been extensively studied. In one study treatment of the CaCo-2 cells with 25 μM of resveratrol caused a 70% growth inhibition. Oral administration of high resveratrol doses in drinking water and diet has been demonstrated to reduce tumour incidence in mice (Athar et al., 2007). The promising results in studies of the effect of resveratrol on colon cancer have led to a clinical trial in which patients with colon cancer receive treatment with resveratrol and correlative laboratory studies will examine its effects directly on colon cancer and normal colonic mucosa. These studies will provide data on the mechanisms of resveratrol action and provide a foundation for future prevention trials, correlative studies and therapeutic clinical research with resveratrol.

In mice, resveratrol supplementation delays spontaneous mammary tumour development and reduced metastasis (Provinceial et al., 2005). In a population study conducted in Italy, an inverse relationship was observed between resveratrol from grape consumption and breast cancer, but not for resveratrol ingested in wine (La Vecchia & Bosetti, 2006).

Research studies show that drinking a glass of red wine a day may cut a man’s risk of prostate cancer by half, and that the protective effect appears to be strongest against the most aggressive forms of the
disease. It was also seen that men who consumed four or more 4-oz glasses of red wine per week have a 60% lower incidence of the more aggressive types of prostate cancer (http://www.cancer.gov/cancer-topics/factsheet/red-wine-and-cancer-prevention).

However, it is impossible to make definite statements or conclusions on the clinical efficacy in cancer patients because of the great variability and differences of the study designs, small patient numbers, short treatment duration and lack of a standardized drug formulation. Although some results from these clinical studies seem encouraging, reliable or long-term data on tumour recurrence, disease progression and survival are unknown. At present, there is no convincing clinical proof or evidence that resveratrol might be used in an attempt to cure cancer. Clinical trials in phase I are being conducted in healthy people in order first to determine the concentration of resveratrol and its metabolites in the plasma, urine, and feces of healthy participants; second, to correlate dose with systemic concentration of this drug and its metabolites in these participants; and thirdly, to determine the safety of this drug in these participants (http://www.cancer.gov/clinicaltrials/CCUM-2004-0535).

2.3.4. Antidiabetic activity

Data in the literature indicate that resveratrol may play a role in the prevention of diabetes and diabetic complications (Harikumar & Aggarwal, 2008). An in vivo experiment revealed that resveratrol, administered to normal rats at the dose of 50 mg/kg body weight, diminished blood insulin concentrations at 30 min, without concomitant changes in glycemia. These findings suggest the direct insulin-suppressive action of resveratrol in the rat (Szkudelski, 2008).

2.3.5. Neuroprotective activity

Neural dysfunction and metabolic imbalances underlie many progressive neurodegenerative conditions such as Alzheimer’s, Huntington’s and Parkinson’s diseases (Sinclair, 2005). Resveratrol is capable of penetrating the blood–brain barrier and exerts strong neuroprotective effects, even at low doses. Resveratrol has been shown to combat the neuronal dysfunction caused in Huntington’s and Alzheimer’s diseases, through the SIRT1 pathway (Parker et al., 2005). The same authors showed that only 500 nM per day, an amount which is provided in one glass of red wine, is needed to protect neurones. The prevention of Parkinson’s disease is based on the scavenging mechanism performed by resveratrol (Karlsson, Emgard, Brundin, & Burkitt, 2000). The efficacy of resveratrol against various different mechanisms has recently been confirmed, and resveratrol has been shown to be potentially useful in protecting against brain damage following cerebral ischemia (Dong et al., 2008).

It is worth mentioning an interesting study developed by Karuppagounder et al. (2009). They fed mice with clinically feasible dosages of resveratrol for 45 days. Neither resveratrol nor its conjugated metabolites were detectable in the brain. Nevertheless, resveratrol diminished plaque formation in a region specific manner. The largest reductions in the percent area occupied by plaques were observed in medial cortex (−48%), striatum (−89%) and hypothalamus (−90%). The changes occurred without detectable activation of SIRT-1 or alterations in APP processing. However, brain glutathione declined 21% and brain cysteine increased 54%, which may be linked to the diminished plaque formation. This study supports the concept that onset of neurodegenerative disease may be delayed or mitigated with the use of dietary chemo-preventive agents that protect against β-amyloid plaque formation and oxidative damage.

2.3.6. Anti-aging activity

Resveratrol extends the lifespan of S. cerevisiae, Caenorhabditis elegans and Drosophila melanogaster, as well as species of short-lived fish through activation of the sirtuin pathways (Howitz et al., 2003; Valenzano et al., 2006; Wood et al., 2004). More recently, Baur et al. (2006) have shown that resveratrol shifts the physiology of middle-aged mice on high-calorie diet towards that of mice on standard diet and significantly increases their survival. Specifically, studies in mice have shown that obese animals whose diet was supplemented with resveratrol not only lived longer, but were more active and produced fewer cases of the negative effects of a high-calorie diet; this diet also reduced insulin-like growth factor-1 levels, increased the number of mitochondria, and improved motor function.

3. Hydroxytyrosol

3.1. Structure and concentration in wine

Hydroxytyrosol is a phenyl ethyl alcohol, 2-(3,4-dihydroxy-phenyl) ethanol (3,4-DHPEA) (Fig. 1b). The main source of hydroxytyrosol in the diet is virgin olive oil, being present, mainly as secoiridoid derivatives or as acetate and free form (Mateos et al., 2001). Hydroxytyrosol and its derivatives arise from oleuropein
Although factors such as variety, olive fruit maturity, olive oil processing or even agronomic factors strongly determine the final amount of phenolic compounds detected in virgin olive oil, concentrations between 100 and 600 mg/kg have been quantified, of which approximately half of this amount correspond to hydroxytyrosol and its derivatives (Brenes, García, García, Rios, & Garrido, 1999; Tripoli et al., 2005).

Wine seems to be another important source of hydroxytyrosol in our diet. It was firstly detected in Italian wines (Di Tommaso, Calabrese, & Rotillo, 1998). Some authors described higher concentrations in red wines (3.66–4.20 mg/l) than white ones (1.72–1.92 mg/l). They hypothesized the formation of hydroxytyrosol from tyrosol during alcoholic fermentation. Later, hydroxytyrosol was detected in Greek wines (Proestos et al., 2005), while simultaneously new Italian findings also confirmed the presence of hydroxytyrosol in their wines (Boselli, Minardi, Giomo, & Frega, 2006; Dudley et al., 2008).

Minuti, Pellegrino, and Teseo (2006) evaluated different extraction processes of hydroxytyrosol from wines, quantifying concentrations ranging from 1.8 to 3.1 mg/l in red wine (Table 2). All of the above described evidences render wines as an important source of hydroxytyrosol in diet.

### Table 2

<table>
<thead>
<tr>
<th>Hydroxytyrosol and tyrosol contents in wine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxytyrosol (mg/l)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>White wine</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Red wine</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Aged red wine</td>
</tr>
</tbody>
</table>

Nq, no quantified.

Urinary recoveries as high as 80% of the ingested amounts of hydroxytyrosol have been reported (Miró–Casas et al., 2003a, 2003b). Over 90% of the urinary metabolites were conjugated (Caruso et al., 2001; Miró–Casas et al., 2001, 2003a, 2003b; Visioli et al., 2000; Vissers et al., 2002). Mainly glucuronidated metabolites, yet free phenols and methylconjugates, with or without glucuronidation, were also excreted in human urine. Sulfon conjugates of hydroxytyrosol, and other metabolites such as 3,4-dihydroxyphenylethylacetate and 3,4-dihydroxyphenylacetic acid were also identified after oral or intravenous dosing of hydroxytyrosol in animal experiments (D‘Angelo et al., 2001; Tuck & Hayball, 2002). Analyses of urinary samples from ten healthy volunteers have been evaluated by LC-ESI-TOF-MS after a high intake of extra-virgin olive oil was carried out. More than 50 metabolites were tentatively identified, where methylation and glucuronidation were the most common metabolic reactions. Additionally, kinetic studies were conducted on the metabolites identified, and the highest level of the most compounds was detected after 2 h of olive oil administration (García–Villalba et al., 2010).

To identify associations between polyphenol intake and health and disease outcomes in cohort studies, it is important to identify biomarkers of intake for the various compounds commonly consumed as part of the diet. In this sense, hydroxytyrosol could be considered as a useful biomarker of intake since its recovery yield in urine showed a high correlation with the dose ingested (Pérez–Jiménez et al., 2010).

The knowledge of structural form of olive oil polyphenols within the peripheral circulation (and other target tissues) is essential in order to obtain additional information about their mechanism of action in vivo (Kroon et al., 2004). In this sense, the radical scavenging potencies of the conjugates detected after hydroxytyrosol metabolism have also been investigated using the DPPH assay. The results showed out more activity for the monoglucuronide conjugate in comparison with its precursor, hydroxytyrosol; while the monosulphate conjugate did not show significant radical scavenging activity (Tuck & Hayball, 2002). In contrast with these results, the antioxidant activity of monoglucuronide conjugates was recently evaluated by DPPH assay and inhibition of Cu-mediated LDL oxidation at physiological concentrations (0.01–10 μM), without observing significant antioxidant activity at the concentration tested (Khymenets et al., 2010).

Although the conjugation suffered by hydroxytyrosol after its absorption changes the in vivo antioxidant activity in comparison with the unmodified compound, it is not possible to extrapolate these results to an in vivo situation. Therefore, it is important to identify target tissues of hydroxytyrosol. A study of the tissue distribution after intravenous administration of radioactive hydroxytyrosol in rats demonstrated a fast and extensive uptake of this molecule by the organs and tissues investigated, such as skeletal muscle, liver, lungs or heart, with a preferential renal uptake (D‘Angelo et al., 2001). Moreover, 90% of the administered radioactivity was detected in urine, collected up to 5 h after injection mainly in its conjugated forms, while about 5% was found in feces and gastrointestinal content.

Regarding bioavailability of hydroxytyrosol containing wine, a recent study compared the hydroxytyrosol pharmacokinetics after moderate doses of wine and olive oil in healthy volunteers (De la Torre et al., 2006). It was observed that the daily ingestion of 250 ml of wine leads to plasma concentrations of about 8 ng/ml of hydroxytyrosol. Likewise, the results showed a higher urinary recovery of hydroxytyrosol after red wine administration in spite of the fivefold differences in the doses administered (0.35 mg for red wine and 1.7 mg for olive oil). These authors hypothesized the interaction between ethanol and dopamine after red wine ingestion, leading to the formation of hydroxytyrosol. In agreement with these findings, Schröder et al. (2009) confirmed that...
wine is an important source of hydroxytyrosol and they suggested alcohol as an indirect promoter of endogenous hydroxytyrosol generation.

Finally, as regards toxic effects of hydroxytyrosol, few studies have been carried out to evaluate its toxicity. D'Angelo et al. (2001) found that the oral administration of hydroxytyrosol to rats did not show any sign of toxicity up to 2 g/kg body weight. Recently Soni, Burdock, Christian, Bitler, and Crea (2006) performed toxicity studies with an extract rich in hydroxytyrosol (50–70% of weight extract). It did not cause toxicity at levels up to 2000 mg/kg/day. In the in vivo micronucleus assay, oral exposure of rats to hydroxytyrosol at dose levels up to 5000 mg/kg/day for 29 days did not induce increase in polychromatic erythrocytes in bone marrow. The consumption of hydroxytyrosol is considered to be safe at levels up to 20 mg/kg/day according to the available studies of the extract and polyphenols.

3.3. Health-promoting properties

3.3.1. General

In this section the health benefits of hydroxytyrosol have been reviewed. As previously commented, few studies have been developed on the wine matrix. The majorities of these studies are actually performed on olive oil matrix since olive oil is the main source of hydroxytyrosol in diet.

3.3.2. Antioxidant activity

The phenolic antioxidant activity of hydroxytyrosol has been studied exhaustively using many different techniques in abiotic model systems where reactive oxygen species (ROS) and other radicals are generated by a variety of agents. When compared with other phenolic compounds, including tyrosol, hydroxytyrosol showed a much more effective antioxidant character. Evidently, antioxidant activity does not always correlate in different assay methods, and the catechol (o-dihydroxy) structure is not always required for antioxidant activity. However, as a general rule, the ortho-dihydroxy structure occurring in hydroxytyrosol and oleuropein have the highest antioxidant activity, followed by 4-O-mono-hydroxy compounds (ligstroside and tyrosol), and 3-O-hydroxy- substituted catechols; and all of these compounds are stronger antioxidants than either ascorbic acid or alpha-tocopherol (De Pinedo, Peñalver, & Morales, 2007; Mateos, Domínguez, Espartero, & Cert, 2003; Tuck & Hayball, 2002).

Hydroxytyrosol has been proven to have antioxidant activity in vitro, scavenging peroxyl, hydroxyl and other free radicals, reactive nitrogen species, and superoxide anions, breaking peroxidative chain reactions and preventing metal ion catalyzed production of reactive oxygen species (ROS) (Cornwell & Jiyan, 2008; Tripoli et al., 2005).

Although biological actions of phenolic compounds have been commonly related to its free radical scavenging activity (Goya, Mateos, & Bravo, 2007), current evidences strongly support that hydroxytyrosol may also offer an indirect protection by increasing the endogenous defence systems (Jemai, Bouaziz, Fki, El Feki, & Sayadi, 2008; Olivera–López et al., 2008). In fact, Martin et al. (2010) confirmed this additional mechanism of action to prevent oxidative stress damage by inducing antioxidant enzymes, which act as critically important regulators in cell protection from oxidative stress and chemical-induced damage by controlling the intracellular redox status.

Considering the antioxidant inherent activity of hydroxytyrosol, it could be involved in the prevention of pathologies, such as cancer, cardiovascular disease, neurodegenerative disorders, diabetes, inflammation, etc., where etiology and progression have been related to ROS-mediated tissue injury. Biomarkers of oxidative damage based on the measurement of various relatively stable oxidation products, arising from DNA damage, LDL oxidation or reduced glutathione (GSH) among others, have permitted to confirm the extensive biological activity detailed for this compound (Mateos & Bravo, 2007).

3.3.3. Cardioprotective capacity

Health effects of virgin olive oil intake in cardiovascular disease are greatly attributed to its high content in monounsaturated fatty acid (MUFA), but also to phenolic compounds such as hydroxytyrosol. In this sense, the EUROLIVE study held in 200 healthy male volunteers, demonstrated that the intake of three kinds of virgin olive oil with different phenolic content increased HDL-cholesterol and reduced lipid oxidative damage in a dose-dependent manner (Covas et al., 2006). These results are in agreement with those previously reported by Marrugat et al. (2004), who observed that the intake of high-phenolic content virgin olive oil decreased lipid oxidative damage. Furthermore, Visioli, Wolfram, Richard, Abdullah, and Crea (2009) recently showed that olive oil phenolic compounds obtained from olive mill waste water, in which hydroxytyrosol is the most bioactive component, increased total plasma glutathione levels when administered to 98 healthy volunteers. These results pointed out the capacity of hydroxytyrosol to prevent LDL oxidation and improve the lipid profile after its continuous consumption.

In addition to the ability of hydroxytyrosol to prevent LDL oxidation, hydroxytyrosol showed a beneficial effect on platelet function. The influence of phenolic compounds from virgin olive oil on cell adhesion molecules is uncertain. Pacheco et al. (2007) observed a postprandial decrease in molecular cell adhesion after intake of virgin olive oil compared with refined olive oil. However, consumption of virgin olive oil rich in phenolic compounds by stable coronary disease patients did not significantly influenced VCAM-1 and ICAM-1 plasma concentration (Fitó et al., 2008). Related to the benefits of hydroxytyrosol on platelet function, Dell’Aglì et al. (2008) confirmed its ability to inhibit platelet aggregation in vitro. Otherwise, the intake of high-phenolic content virgin olive oil by 21 hypercholesterolemic volunteers, decreased plasminogen activator inhibitor-1 and factor VII, associated with changes in the postprandial hemostatic profile, leading to a less thrombogenic state.

Concerning the development of atherosclerotic lesions, a study carried out in hyperlipidemic rabbits fed with a diet supplemented with hydroxytyrosol, showed an improvement of the antioxidant status and reduction of the size of atherosclerotic lesions when compared with control animals (González–Santiago et al., 2006).

It should be mentioned an interesting study in which the cardioprotective effects of hydroxytyrosol have been compared with others potent antioxidants, in addition to white and red wine. Rats treated for 14 days with hydroxytyrosol (2.5 mg/kg), tyrosol (2.5 mg/kg), resveratrol (2.5 mg/kg), as well as white wine and red wine, were sacrificed to isolate cardiomyocytes cells. The most surprising finding is the ability of white wine to induce the longevity proteins that in comparison with red wine and the rest of antioxidants showed the following order of activity: white wine > resveratrol > tyrosol > hydroxytyrosol > red wine. However, the cardioprotection exerted by reduction of infarct size and cardiomyocytes apoptosis followed a different pattern: resveratrol > red wine > hydroxytyrosol > white wine > tyrosol, suggesting the existence of different signaling mechanisms for the induction of longevity and survival (Mukherjee, Lekli, Gurusamy, Bertelli, & Das, 2009).

Finally, elevated concentrations of inflammation markers are associated with increased cardiovascular risk. Thus, reduction of tromboxane B2 and leukotriene B4 levels, as proinflammatory agents, has been repeatedly reported in intervention studies (Bogani, Galli, Villa, & Visioli, 2007; Oubilha, Sánchez–Muniz, Ró
denas, & Cuesta, 2001; Visioli et al., 2005). Concerning pro-inflammatory cytokine and C-reactive protein, they significantly improved after the consumption of virgin olive oil rich in phenolic compounds in stable coronary disease patients (Fité et al., 2008). Recently the activity of hydroxytyrosol prepared in a mixture of about 20% of this polyphenol (HT-20) in carrageenan-induced acute inflammation rats was evaluated. The rodents received different dosages (100, 250 and 500 mg/kg of body weight) by gavage of HT-20. This product significantly inhibited both the acute inflammation and the pain associated with carrageenan administration. The analgesic action of HT-20 was not in a dose-dependant manner and it was able to decrease pro-inflammatory cytokines IL-1beta and TNF-alpha, but not to increase the antiinflammatory cytokine mRNA expression of IL-10 (Gong et al., 2008).

3.3.4. Anticancer activity

The epidemiological studies carried out so far show evidence that olive oil consumption may reduce the risk of breast cancer (Trichopoulou & Dilis, 2007). Moreover, some intervention studies showed a significant improvement in makers of oxidative DNA damage after the intake of virgin olive oil, suggesting its ability to prevent some types of cancer. In the EUROLiVe substudy (Machowetz et al., 2007) the intake of olive oil reduced urinary 8-oxodeoxyguanosine levels, regardless of the phenolic content. In addition, the consumption of phenolic rich virgin olive oil could be responsible for the reduction of DNA damage in peripheral blood lymphocytes in postmenopausal women (Salvini et al., 2006).

The connection between chronic inflammation and tumour growth has received much attention and it is estimated that inflammation contributes to 15–20% of all cancers (Marx, 2004). In this sense, the described antiinflammatory activity in the cardioprotective capacity section for hydroxytyrosol implicitly demonstrates its potential anticarcinogenic activity. Indeed, hydroxytyrosol blocks the transcription of the enzymes COX-2 and 5-lipooxygenase, reducing the prostaglandin E2 synthesis and, thus, the chronic influence associated with diseases such as cancer (Cornwell & Jiyam, 2008). Likewise, Caco-2 cells treatment with olive oil polyphenols exerted anticancer effect by inhibition of COX-2 expression (Corona et al., 2007).

In addition, hydroxytyrosol alters tumour eicosanoid biosynthesis and shows a wide range of antitumour effects, inhibiting proliferation and promoting apoptosis in several human tumour-cell lines through several mechanisms (Corona et al., 2009; Fabiani, Fuccelli, Pieravanti, De Bartolomeo, & Morozzi, 2009; Han, Talorette, Yamada, & Isoda, 2009; Sirianini et al., 2010).

Finally, oxidative DNA damage is prevented by hydroxytyrosol in human blood mononuclear cells and HL60 cells (Fabiani et al., 2008).

3.3.5. Antimicrobial activity

Hydroxytyrosol is able to inhibit or delay the rate of growth of a range of bacteria, microfungi and pathogenic bacteria in humans, the antimicrobial activity of polyphenols containing olive fruit (Fleming, Walter, & Etchells, 1973), olive oil mill waste waters (Capasso et al., 1995), olive leaves (Markin, Duek, & Berdichevsky, 2003) and olive oil (Receli & Robinson, 2002; Radford, Tassou, Nychas, & Board, 1991) has been reported. Medina, de Castro, Romero, and Brenes (2006) showed a strong bactericidal action of hydroxytyrosol against a broad spectrum of microorganisms, and was higher in general against Gram-positive than Gram-negative bacteria. Moreover, it showed bactericidal activity, not only against harmful bacteria of the intestinal microbiota (Clostridium perfringens and Escherichia coli), but also against beneficial microorganisms such as Lactobacillus acidophilus and Bifidobacterium bifidum. Likewise, most of the foodbome pathogens tested (Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica, Yersinia sp., and Shigella sonnei) did not survive after 1 h of contact with this biophenol.

Recently, Brenes, Medina, Romero, and de Castro (2007) compared the antimicrobial activity of olive oil with that reported for foods such as tea, coffee and wine, among others. Results indicated a higher capacity of virgin olive oil than wine to inhibit the growth of pathogenic bacteria. Medina, Brenes, Romero, García, and de Castro (2007) evaluated comparatively the antimicrobial activity of olive oil, vinegar and various beverages, such as wine, against foodborne pathogens. Vinegar and aqueous extracts of virgin olive oil showed the strongest bactericidal activity against all strains tested, closely followed by red and white wines.

On the other hand, extracts from the byproducts of olive oil and wine production, showed high antimicrobial activity against Escherichia coli, Candida albicans, Saccharomices cerevisise, and Bacillus cereus (Serra et al., 2008). The authors suggest that the natural extracts may have important applications in the future as natural antimicrobial agents for the food industry, as well as for medical use. Indeed, this type of preservative has been tested in fish fillets with promising results (Pazos, Alonso, Fernandez–Bolanos, Torres, & Medina, 2006).

In addition, Bisignano et al. (1999) found that hydroxytyrosol has antimicrobial properties against several bacterial strains that are causal agents of intestinal or respiratory tract infections in humans. Likewise, in a more recent in vivo study Glatze et al. (2007) demonstrated that virgin olive oil is more potent than fish oil to reduce septic pulmonary dysfunctions in rats. Moreover, Brenes et al. (2007) showed that hydroxytyrosol has a strong bactericidal activity in vitro against Helicobacter pylory that suggests its potential as a chemopreventive agent for peptic ulcers or gastric cancer.

Finally, hydroxytyrosol, not only shows antibacterial activity, but also antifungal activity against Fusarium sambucinum, Verticillium dahliae and Alternaria solani, as has recently been tested with enriched-hydroxytyrosol extracts (Yangui, Dhouib, Rhouma, & Sayadi, 2009).

3.3.6. Antidiabetic activity

Oxidative stress also plays a role in the pathogenesis of insulin resistance and it has been hypothesized that dietary antioxidants could diminish the risk of diabetes. Therefore, specific dietary strategies may contribute to improve glucose homeostasis and help in the prevention of this disease. In this sense, prospective observational studies and intervention studies support an inverse relationship between Mediterranean diet and insulin resistance (Pauwels, 2009).

Considering that hydroxytyrosol is involved in the prevention of stress oxidative, its effect in alloxa-induced diabetic rats after the consumption of purified compound was evaluated. Results confirmed the ability of hydroxytyrosol to inhibit oxidative stress (Hamden, Allouche, Damak, & Elfeki, 2009; Jemai, El Feki, & Sayadi, 2009) and hyperglycemia (Hamden et al., 2009).

Likewise, polyphenols containing olive leaf extract, such as oleuropein and hydroxytyrosol, reverted the chronic inflammation and oxidative stress that induces the cardiovascular, hepatic, and metabolic symptoms in this rat model of diet-induced obesity and diabetes, without changing blood pressure (Poudyal, Campbell, & Brown, 2010).

Hamden et al. (2010) recently found in diabetic rats an inhibitory action of hydroxytyrosol on pancreatic toxicity after consuming hydroxytyrosol-supplemented diets.

3.3.7. Neuroprotective activity

The importance of olive oil as a major component of the Mediterranean diet to counteract neurodegenerative age-related diseases such as Alzheimer’s and Parkinson’s diseases has been suggested (Berr et al., 2009; Scarmeas, Stern, Tang, Mayeux, &
Luchsinger, 2006). On the one hand, results from the Three-City Study (Berr et al., 2009) revealed that participants with moderate or intensive use of olive oil compared to those who never consume olive oil showed lower odds of cognitive deficit for verbal fluency and visual memory during a 4-year follow-up of 6947 subjects. On the other hand, results published by Scarmeas et al. (2006) from a follow-up study carried out with 2258 subjects showed that higher adherence to the Mediterranean diet is associated with a reduction in the risk of Alzheimer’s dysfunction.

Recent findings about the benefits of hydroxytyrosol to prevent neuronal diseases have proliferated. This hypothesis is supported considering that hydroxytyrosol could cross the blood–brain barrier to appear in the brain. Thus, the measurement of free hydroxytyrosol by liquid chromatography with fluorescence in microdialysates from blood and brain of anesthetized rats permitted to determine the rapid elimination of hydroxytyrosol and its uptake by brain (Wu, Lin, & Tsai, 2009).

The extract enriched in oleuropein has shown neuroprotective activity by forming a non-covalent complex with the Aβ peptide, which is a key hallmark of several neurodegenerative diseases like Alzheimer and Parkinson. Thus, hydroxytyrosol, which is the principal degradation product of oleuropein, has been suggested as the potential neuroprotective compound (Bazoti, Bergquist, Markides, & Tsarbopoulos, 2006). The neuroprotective effect of hydroxytyrosol was recently tested in a model of hypoxia-reoxygenation in rat brain slices, in vitro and in vivo. Hydroxytyrosol significantly inhibited LDL efflux in a dose-dependant manner, providing a preliminary basis for further study as potential neuroprotective compounds (González–Correa et al., 2008).

4. Melatonin

4.1. Structure and concentration in wine

Melatonin is an indolamine (N-acetyl-5-methoxytryptamine) (Fig. 1c). This neurohormone was discovered in the pineal gland and it is also produced as secondary metabolite in plants.

Melatonin has been shown to be synthesized from tryptophan via 5-hydroxytryptophan, serotonin and N-acetylsertotonin and to be metabolized by deacetylation to 5-methoxytryptamine (Fig. 4). Moreover, melatonin can also be formed by O-methylation of serotonin followed by N-acetylation of 5-methoxytryptamine in yeast (Hardeland, Reiter, Poeggeler, & Tan, 1993; Sprenger, Harde-

Fig. 4. Biosynthesis pathway of melatonin (Iriti et al., 2006).

Medicinal herbs such as Tanacetum parthenium or Hypericum perforatum are rich in melatonin (Murch, Simmons, & Saxena, 1997), but they cannot be considered as normal dietary sources. Melatonin has been reported in edible seeds, such as rice and sweet corn (Hattori et al., 1995; Manchester et al., 2000), roots, leaves and fruits of a considerable variety of plants. Indeed, melatonin is present in strawberries, kiwis, pineapples, bananas and apples (Paredes, Korkmaz, Manchester, Tan, & Reiter, 2009). In fact, the occurrence of melatonin in different varieties of strawberries and tomatoes has recently been reported (Stürtz, Cerezo, Cantos, & García–Parrilla, in press). The amounts range from 1.38 to 11.26 ng/g in strawberry and from 4.11 to 114.52 ng/g in tomatoes. The consumption of fresh fruits containing ascorbic acid, which protects melatonin from oxidation, was a positive factor in overall melatonin dietetic intake.

Melatonin has also been found in olive oil at higher levels in extra virgin olive oil than in refined olive or sunflower oil samples (De la Puerta et al., 2007). In addition, melatonin has recently been reported in grapes and wines. Iriti, Rossoni, and Faoro (2006) found melatonin in different grape varieties: Nebbioso, Croatina, Sangiovese, Merlot, Marzemino, Cabernet franc, Cabernet sauvignon and Barbera. The melatonin concentration ranged from 0.005 to 0.9 ng/g. Melatonin was detected by mass spectrometry in Merlot ripening grapes (Murch, Hall, Le, & Saxena, 2010). The increase of indolamines as melatonin and serotonin during veraison of grapes from different vineyards reveals a role in plant physiology. Melatonin has also been reported in wine (0.4–0.5 ng/ml) as determined by HPLC-Fluorescence analysis (Mercolini et al., 2008). Melatonin was assessed in Malbec (0.24 ng/ml), Cabernet Sauvignon (0.32 ng/ml) and Chardonnay (0.16 ng/ml) by means of capillary electropho-

The European Food Safety Authority has recently accepted the scientific evidence of health claims in relation to melatonin and alleviation of subjective feelings of jet lag, reduction of sleep onset latency, and contribution to sleep quality. The melatonin dose should be between 0.5 and 5 mg and should be taken close to be...
dtime on the first day (and any subsequent day) of travel and on the following few days after arrival at the destination. The target population is the general population (EFSA, 2010).

It has already been demonstrated that melatonin is well absorbed after oral administration. After an oral intake of 250 μg plasmatic concentration varies from 155 pg/ml to 720 pg/ml, depending on the sex of the volunteer. Absorption is affected by parameters that can vary widely from one subject to another. However, other pharmacokinetic variables such as elimination and distribution half-life seem to be constant from one subject to another. Bioavailability reported values vary from 22% (Waldhauser et al., 1984) to 8.7% (Fourtillan et al., 2000), or to 33.0% (Di, Kadva, John- ston, & Silman, 1997).

The effect of food containing melatonin on melatonin levels was tested (Hattori et al., 1995). Chickens were fed with corn, rice, beans and milo after the animals had been fasting for 48 h. Doubled daytime melatonin levels were detected. Despite the fact that melatonin content in foods is low, melatonin plasmatic concentra-
tion increased after animals were fed with nuts (Juglans regia) in a reasonable dose (3 g). The dietary intake was 10.5 ng and the plas-
matic concentration increased from 11.5 ± 1.9 to 38.0 ± 4.3 pg/ml (Reiter, Tan, & Maldonado, 2005), and plasma antioxidant capacity determined with the FRAP method simultaneously increased.

In addition to plasmatic levels of melatonin, the melatonin metabo-
lite 6-sulfatoxymelatonin, excreted in urine, is easy to determine. Indeed, a higher excretion of this metabolite in the first morning urine was 16% higher in women with the highest quartile vegetable intake in comparison to the lowest quartile intake (Nag- ata, Nagao, Shibuya, Kashiki, & Shimizu, 2005). Moreover, nutri-
tional and lifestyle have also been correlated with circulating melatonin levels (Dopfel, Schulmeister, & Schernhammer, 2007). An statistical inverse relation among age, smoking and body mass index with urinary 6-sulfatoxymelatonin was found (Schermham-
mer & Hankinson, 2005). In addition to these statistical associa-
tions, a study carried out with rats established a relationship be-
tween melatonin and body weight. Animals were put on a diet to induce obesity. Afterwards, they were treated with melatonin for 3 weeks (30 mg/kg) or pinealectomized. Adipose tissue, weight, insulinemia and glycemia increased in the pinealectomized rats, for 3 weeks (30 mg/kg) or pinealectomized. Adipose tissue, weight, to induce obesity. Afterwards, they were treated with melatonin between melatonin and body weight. Animals were put on a diet 

Table 3
Melatonin content in wines.
<table>
<thead>
<tr>
<th>Bioactive compound (ng/ml)</th>
<th>White wine</th>
<th>Red wine</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melatonin 0.4</td>
<td>0.5</td>
<td>Mercolini et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.3</td>
<td>Stege et al. (2010)</td>
<td></td>
</tr>
<tr>
<td>391</td>
<td>35</td>
<td>Rodríguez–Naranjo et al. (2011a, 2011b)</td>
<td></td>
</tr>
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</table>

total food intake due to an increase in carbohydrate-rich diets. Data reported were consistent for the different melatonin doses employed. However, the authors could not find a consistent pattern for protein-rich diets (Angers, Haddad, Selmaoui, & Thibault, 2003). The hypothesis to explain the obtained results considers the effect of melatonin as a circadian marker. If melatonin marks the night period, rodents involved in the study will eat more. How-

er, the opposite effect is expected in humans (eating less as night starts). Indeed, higher melatonin concentration was found in anor-
exic people (Arendt, Bhanji, Franey, & Mattingly, 1992). The work by Angers et al. (2003) reveals the need to study the effect of mel-
tonin on food intake as being dependent on the dose and time when supplements are taken.

Mustonen, Nieminen, and Hyvarinen (2002) investigated sub-
acute effects of persistent melatonin treatment and continuous light on carbohydrate and fat metabolism of rat liver and kidney. Exogenous melatonin enhanced utilization of liver carbohydrates, but suppressed hepatic lipolysis.

Finally, as regards toxic effects of melatonin few studies have been carried out to evaluate the melatonin toxicity. Melatonin itself turned out to be neither toxic nor mutagenic in high concentra-
tion assays (Anisimov, 2003). However, further studies and clinical trials are needed to verify that melatonin is safe.

4.3. Health-promoting properties

4.3.1. Antioxidant capacity

Melatonin is a significant free radical scavenger and antioxid-
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Indeed, melatonin is able to scavenge H2O2 in a dose–dependent manner. As a result, N(1)-acetyl-N(2)-formyl-5-methoxykynu-
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even reduce blood pressure in males with chronic hypertension (Scheer, Van Montfrans, Van Someren, Mairuhu, & Buijs, 2004).

As an antioxidant melatonin has been found to be particularly effective in reducing free-radical-mediated damage to DNA. Damaged DNA, if it goes unrepaired, may mutate and initiate a tumour.

### 4.3.2. Anticancer activity

Since 1994, when the first paper was published documenting the role of melatonin in apoptosis, the number of reports in this area has rapidly increased (Maestroni, Covacci, & Conti, 1994; Sainz et al., 2003).

Melatonin may play an important role in carcinogenesis, as suggested by substantial laboratory and less direct epidemiologic evidence. Particularly, in experimental animals cancer growth is exaggerated when the animals are repeatedly phase advanced (as occurs during easterly flights) or exposed to light at night (Reiter et al., 2007). If, in fact, physiological levels of melatonin normally restrain tumour growth, the age-associated reduction in melatonin production may be contributory to the increased frequency of cancer in the elderly. There is also some evidence to indicate that the efficacy of melatonin in limiting tumour cell proliferation depends on the time of day of its administration, with melatonin given late in the light phase being more effective (Sauer, Dauchy, & Blask, 2001).

Mechanistically, how melatonin inhibits tumour cell proliferation has partially been defined and it apparently involves a number of mechanisms. Apart from those, it is remarkable that there are also some other actions implied. In oestrogen-receptor-positive human breast cancer cells melatonin is thought to modulate oestrogen receptor expression and transactivation. Other potential mechanisms still include melatonin's ability to reduce angiogenesis in tumours, to delay the G1 to S phase transition in the cell cycle, to improve cellular communication between normal and cancer cells, and to alter the intracellular redox state. Besides inhibiting established tumours, melatonin may also decrease their initiation (Reiter, 2003).

The use of melatonin in humans reduced tumour growth in some cases and prolonged survival of cancer patients compared with individuals given conventional cancer therapy (Lissoni, 2002). And, more importantly, melatonin administration, when combined with standard chemotherapies, often improves the quality of life. This is probably related to melatonin's ability to reduce the toxicity of chemotherapeutic agents and melatonin action synergistically. Apart from the objective benefits achieved in the patients, researchers also commented on probable subjective advantages of using melatonin. Many of these effects include amelioration of hypotension, myelotoxicity, and lymphocytopenia associated with concomitantly prescribed toxic therapeutic regimens. Perhaps the most clinically relevant feature is that patients receiving melatonin achieve and maintain a better performance status and also have less anxiety than those treated without melatonin. (Lissoni, 2000) have also reported substantial beneficial effects of treatment of cancer patients with melatonin analogues as well. The observations reported in all these clinical investigations are encouraging and indicate that melatonin administration was generally deemed to improve patients suffering from a variety of cancers (Lissoni, 2000; Vijayalaxmi, Thomas, Reiter, & Herman, 2002).

### 4.3.3. Immunomodulatory agent

Melatonin exhibits immunomodulatory properties which are mediated via membrane and nuclear receptors (Guerrero & Reiter, 2002). Data were reported on activation of T, B, NK cells and monocyttes, thymocyte proliferation, release of cytokines (IL-1, IL-2, IL-6, IL-12, and IFN), met-enkephalin, other immunomodulators, and antiapoptotic effects, including glucocorticoid antagonism. Signalling mechanisms are only partially understood and some findings are still contradictory (Liu, Ng, & Fung, 2001).

Daily oral melatonin administration in humans increases natural killer (NK) cell activity (Guerrero et al., 2000). Additionally, melatonin reportedly regulates gene expression of several immunomodulatory cytokines including tumour necrosis factor-α (TNFα), transforming growth factor beta (TGFβ) and stem cell factor (SCF) by peritoneal macrophages as well as the levels of interleukin-1β (IL-1β), interferon gamma (INFγ), TNFα and SCF by splenocytes (Liu et al., 2001). Melatonin is also a potent inhibitor of apoptosis in the immune cell (Reiter, 2003).

The fact that melatonin is generally considered to be immunostimulatory raises the question that whether it should be taken by individuals suffering from an autoimmune disease. To date, the information is meagre regarding this issue, although in one case of Crohn's disease the condition of excessive immune reactivity of the gut wall was aggravated by melatonin. Whether this will be a general finding in autoimmune diseases, however, remains to be established (Reiter, 2003).

### 4.3.4. Neuroprotective activity

A special, but important, aspect is melatonin's role in neuroprotection. Melatonin has been tested in sleep disorders. It generally reduces sleep latency and improves sleep especially when circadian phasing is disturbed. In the latter case, this was particularly effective in patients with neurodegenerative diseases (Srinivasan et al., 2005). Numerous attempts have been made or are under current investigation to mitigate neurodegenerative diseases, such as Alzheimer's, Parkinson's, and Huntington's diseases and amyotrophic lateral sclerosis. Melatonin was shown to inhibit Aβ fibrillogenesis (Asayama et al., 2003; Harderland, Pandi-Perumal, & Cardinai, 2006). It has recently been shown that melatonin (200 mg/kg) reduced edema in impacted striatum versus traumatic brain injury (Kabadi & Maher, 2010).

### 5. Conclusions

The Mediterranean diet has become recognised as a model diet for preventing several serious diseases and cardiac disease in particular. Wine seems to be a key component in this diet and a moderate, regular consumption of wine (two glasses of red wine per day) is recommended.

Resveratrol, hydroxytyrosol and melatonin are three compounds naturally present in wine. They could act synergically to ensure a higher cytoprotective effect against oxidative stress, thus further supporting the hypothesis that health benefits of Mediterranean diet are partly due to wine.

Wine comes in a wide variety of styles (varieties, winemaking, storage conditions, etc.), and therefore they contain quite different bioactive compounds. On average a service of red wine (200 ml) could provide 0.38 mg of resveratrol, 0.45 mg of hydroxytyrosol and 61.4 µg of melatonin, apart from other important bioactive compounds.

It is impossible to make a definitive statement or conclusion about the real effect of these molecules on health since human studies are still in initial stages and also because there is too much variability in the study designs. What can be said is that these molecules look promising in the field of medicine.

However, there are still some issues that need to be addressed. How much resveratrol, hydroxytyrosol and melatonin can be taken and recovered from the organism? How active are the metabolites derived from them? How does the type of meal consumed in association with the ingestion of red wine influence the bioavailability in humans? These are all questions still to be answered.
References


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