

Review

Biological Activities of Phenolic Compounds Present in Virgin Olive Oil

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Abstract: The Mediterranean diet is associated with a lower incidence of atherosclerosis, cardiovascular disease, neurodegenerative diseases and certain types of cancer. The apparent health benefits have been partially ascribed to the dietary consumption of virgin olive oil by Mediterranean populations. Much research has focused on the biologically active phenolic compounds naturally present in virgin olive oils to aid in explaining reduced mortality and morbidity experienced by people consuming a traditional Mediterranean diet. Studies (human, animal, *in vivo* and *in vitro*) have demonstrated that olive oil phenolic compounds have positive effects on certain physiological parameters, such as plasma lipoproteins, oxidative damage, inflammatory markers, platelet and cellular function, antimicrobial activity and bone health. This paper summarizes current knowledge on the bioavailability and biological activities of olive oil phenolic compounds.

Keywords: virgin olive oil; olive oil phenolic compounds; health benefits

Abbreviations: ADDL, beta-amyloid oligomers; CHD, coronary heart disease; COX-1, cyclooxygenase-1; COX-2, cyclooxygenase-2; CRP, C-reactive protein; CVD, cardiovascular disease; DNA, deoxyribonucleic acid; FVII, factor VII; g, gram; GSSG, glutathionedissulfide; GSH, reduced glutathione; GSH-Px, glutathione peroxidase; HDL-C, high density lipoprotein cholesterol; IL-6, interleukin-6; LDL, low density lipoprotein; LDL-C, low density lipoprotein cholesterol; LPO, lipid peroxidation; LTB₄, leukotriene B₄; MUFA, monounsaturated fatty acid; μM, micromolar; μg, microgram; oxLDL, low density lipoprotein oxidation; 8-oxo-dg, 8-oxo-2'-deoxyguanosine; PAI-1, plasminogen

activator inhibitor-1; ROS, reactive oxygen species; sICAM-1, soluble intercellular molecules; sVCAM-1, soluble vascular adhesion molecules; Tau, microtubule-associated protein; TC, total cholesterol; TG, triglyceride; TXB₂, thromboxane B2

1. Introduction

A lower prevalence of non-communicable diseases such as cardiovascular disease and certain types of cancers have been demonstrated in countries residing in the Mediterranean region in comparison to other parts of the world [1–9]. This lowered incidence has been partially attributed to the regular intake of virgin olive oil as part of a traditional Mediterranean diet [3,5,9,10–17]. Dietary consumption of virgin olive oil in a Mediterranean diet typically ranges between 25–50 mL per day [18].

Virgin olive oil is produced from the first and second pressings of the olive fruit by the cold-pressing method (where no chemicals and only a small amount of heat are applied) and is composed of a glycerol fraction (making up 90–99% of the olive fruit) and a non-glycerol or unsaponifiable fraction (making up 0.4–5% of the olive fruit) which contains phenolic compounds [19]. Historically, the beneficial health effects of virgin olive oil intake were attributed to the glycerol fraction with its high concentration of monounsaturated fatty acids (MUFAs) (particularly oleic acid) [19]. However, several seed oils (including sunflower, soybean, and rapeseed) containing high quantities of MUFAs are ineffective in beneficially altering chronic disease risk factors [20,21]. Therefore, a substantial number of investigations examining the biological actions of olive oil phenolic compounds in the unsaponifiable fraction have been conducted. In this paper, the term virgin olive oil will be used to describe both extra virgin and virgin olive oil.

Studies conducted thus far (including human, animal, *in vivo* and *in vitro*) have demonstrated that olive oil phenolic compounds have positive effects on various physiological biomarkers, implicating phenolic compounds as partially responsible for health benefits associated with the Mediterranean diet [3,5,22–27]. Furthermore, olive oil phenolic compounds have been shown to be highly bioavailable, reinforcing their potential health promoting properties [28–33].

The phenolic fraction of virgin olive oil is heterogeneous, with at least 36 structurally distinct phenolic compounds identified. Variation in the phenolic concentration exists between differing virgin olive oils due to numerous factors including: variety of the olive fruit [34–42], region in which the olive fruit is grown [37], agricultural techniques used to cultivate the olive fruit [34,43,44], maturity of the olive fruit at harvest [35,39,44–48], and olive oil extraction, processing, storage methods and time since harvest [38,45,49–57]. Cooking methods have also been shown to alter phenolic concentrations in virgin olive oil [58,59,60]. Finally, research has shown that the analytical method used to quantify the concentration of phenolic compounds present in virgin olive oil has an influence on the reported concentration [61]. The factors mentioned above are not discussed in the current paper. For an extensive review on the matter, please see the paper by Cicerale and colleagues [62]. Therefore, the objective of the current paper was to review the bioavailability of olive oil phenolic compounds and the biological activities associated with them.

2. Bioavailability of Olive Oil Phenolic Compounds

The bioavailability of a compound refers to the degree in which it is extracted from a food matrix and absorbed by the body [63]. The majority of research regarding the bioavailability of olive oil phenolic compounds has focused on three major phenolics: hydroxytyrosol, tyrosol, and oleuropein, and in general, phenolics from virgin olive oil have been demonstrated to be readily bioavailable.

Research has shown that the phenolic compounds, hydroxytyrosol and tyrosol are absorbed after ingestion in a dose-dependent manner [30,64,65]. Tuck and colleagues [66] demonstrated increased bioavailability of hydroxytyrosol and tyrosol when administered as an olive oil solution compared to an aqueous solution. The differences in bioavailability have been suggested to be due to the high antioxidant content of virgin olive oil compared to water and this high antioxidant content may have protected the breakdown of phenolics in the gastrointestinal tract prior to absorption [66]. A further study found that absorption of administered ligstroside-aglycone, hydroxytyrosol, tyrosol, and oleuropein-aglycone was as high as 55–66% in humans [32]. Finally, oleuropein was demonstrated to be somewhat absorbed from isolated perfused rat intestine [67]. The mechanism by which absorption occurs with regards to olive oil phenolic compounds remains unclear. However, the different polarities of the various phenolics has been postulated to play a role in the absorption of these compounds [32]. For instance, the phenolics tyrosol and hydroxytyrosol are polar compounds and their absorption has been postulated to occur via passive diffusion [68]. The polar but larger phenolic, oleuropein-glycoside may be absorbed via a different mechanism to tyrosol and hydroxytyrosol. It has been proposed that oleuropein-glycoside may diffuse through the lipid bilayer of the epithelial cell membrane and be absorbed via a glucose transporter. Two additional mechanisms for oleuropein-glycoside absorption are potentially via the paracellular route or transcellular passive diffusion [67]. The phenolics, oleuropein and ligstroside-aglycones are less polar and currently there is no data available on their mechanism of absorption. Further research is required to substantiate the mechanisms of absorption for these phenolics and further investigate the mechanisms for other phenolic compounds.

Studies examining the quantity of phenolics excreted have also been carried out. A low quantity of phenolics present in urine after ingestion would indicate that these phenolics are readily absorbed. Excreted phenolics (mainly in the form of hydroxytyrosol and tyrosol) were determined to be 5–16% of the total ingested [32]. Excretion of approximately 24% of administered tyrosol was demonstrated in a study by Miro-Cases and colleagues [33]. Finally, Visioli and colleagues [30] reported the excretion of administered hydroxytyrosol and tyrosol to be between 30–60% and 20–22% of the total ingested by human subjects, respectively. The above findings demonstrate that humans absorb a significant portion (~40–95%, using hydroxytyrosol and tyrosol as proxy) of the dietary olive oil phenolic compounds they consume [32]. As most of the data is only based on three phenolics, more research is required on the excretion of other key phenolics in virgin olive oil.

The metabolism of olive oil phenolic compounds is important in determining their availability. If phenolics are broken down and converted to other phenolics this may have a notable effect on their bioavailability. Phenolic compounds, oleuropein-glycoside and oleuropein and ligstroside-aglycones are converted to hydroxytyrosol or tyrosol and excreted in urine [32]. Hydroxytyrosol and tyrosol themselves are sometimes conjugated to glucuronic acid and excreted in urine as glucuronides [30,32,65,69]. However, further work is needed in this area.

3. Olive Oil Phenolic Compounds and Health

Human and animal research have shown that olive oil phenolic compounds possess important biological activities that may exert a preventative effect in regards to the development of chronic degenerative diseases. Figure 1 demonstrates the biological activities exerted by olive oil phenolic compounds. Table 1 briefly summarizes the findings of several human studies that have investigated the biological activities of olive oil phenolic compounds.

Figure 1. Biological activities of olive oil phenolic compounds (adapted from Cicerale *et al.* [62]).

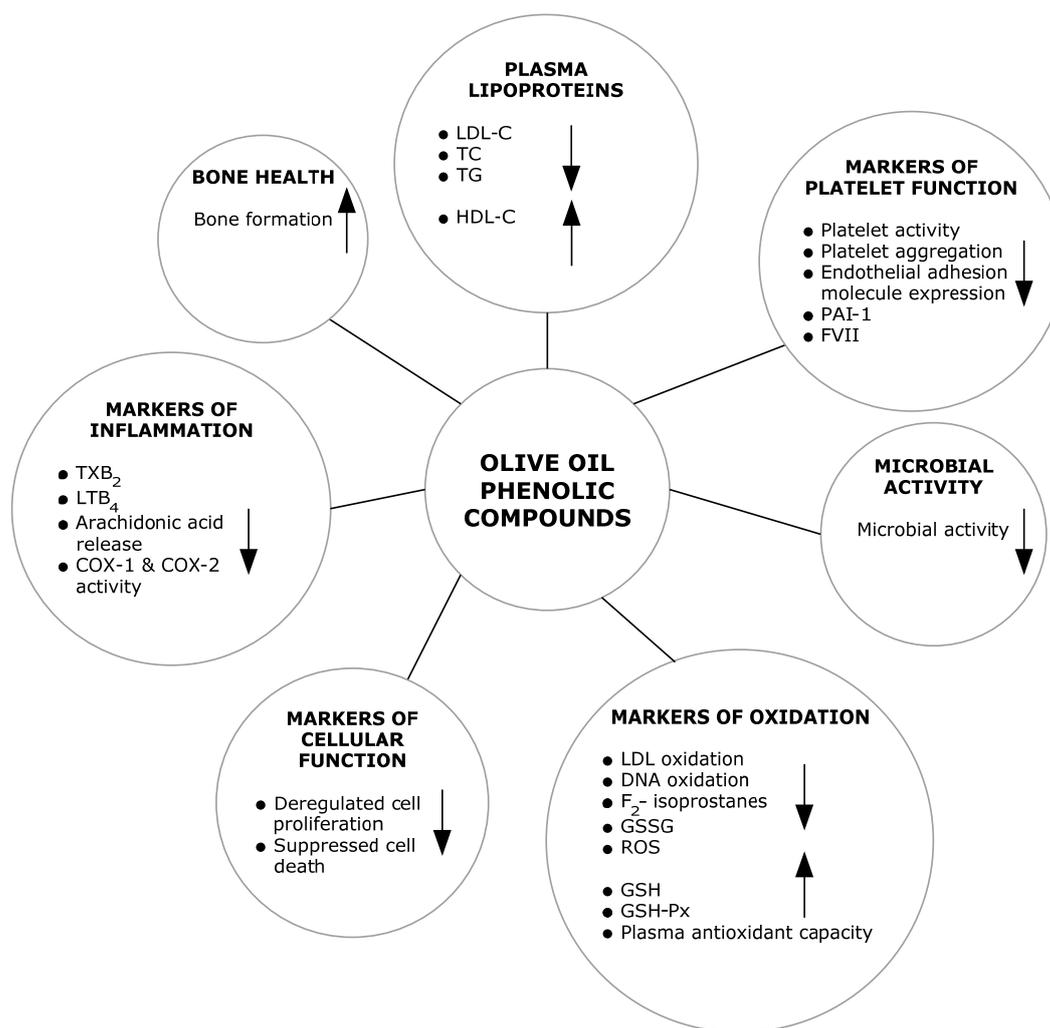


Table 1. Randomized, crossover, controlled, human studies on the effect of olive oil phenolic compounds on biomarkers of health (adapted from Cicerale *et al.* [62]).

Treatment	Subject number	Olive oil phenolic concentration	Study design	Investigated biomarker	Key findings	Ref.
High phenolic concentration vs. low phenolic concentration olive oil	28 coronary heart disease subjects	161 vs. 14.67 mg/kg	3 week, crossover	IL-6, C-reactive protein, sICAM-1, sVCAM-1 and plasma lipids	Interleukin-6 and C-reactive protein decreased after phenol-rich olive oil consumption. However, no changes in soluble intercellular (sICAM-1) and vascular adhesion (sVCAM-1) molecules and lipid profile were observed.	[70]
High phenolic concentration vs. low phenolic concentration-enriched breakfast	21 hypercholesterolemic subjects	400 vs. 80 mg/kg	Acute dose, crossover	FVIIa and PAI-1	Concentrations of FVIIa increased less and PAI-1 activity decreased more after the high phenolic breakfast than after the low phenolic breakfast.	[71]
High phenolic concentration vs. moderate phenolic concentration vs. poor phenolic concentration olive oil	30 healthy subjects	825 vs. 370 vs. 0 μ mol CAE/kg	3 week, crossover	Plasma lipids and oxLDL	An increase in phenolic content of LDL-C and decrease in oxLDL was noted after consumption of oil rich in phenolic compounds.	[72]
High phenolic concentration vs. low phenolic concentration olive oil vs. corn oil	12 healthy subjects	607 vs. 16 vs. 0 mg/kg	Acute dose, crossover	Plasma TXB ₂ , plasma LTB ₄ and plasma antioxidant capacity	Decrease in TXB ₂ and LTB ₄ with increasing phenolic content of olive oil and concomitant increase in plasma antioxidant capacity with increased phenolic content of olive oil.	[73]
High phenolic concentration vs. low phenolic concentration olive oil	10 healthy subjects	592 vs. 147 mg/kg	8 week, crossover	Oxidative DNA damage and plasma antioxidant capacity	A reduction in DNA damage with the consumption of a phenol-rich olive oil diet was demonstrated. No difference was seen in plasma antioxidant capacity.	[74]
High phenolic concentration vs. moderate phenolic concentration vs. low phenolic concentration olive oil	200 healthy subjects	366 vs. 164 vs. 2.7 mg/kg	3 week, crossover	Plasma lipids, plasma oxLDL, plasma F2-isoprostanes, GSH and GSSG	A linear increase in HDL-C was observed for low-, medium-, and high phenolic olive oil. Furthermore, TC to HDL-C ratio decreased linearly with the increasing phenolic content of the olive oil. OxLDL decreased linearly with increasing phenolic content of the olive oil and TG levels decreased for all olive oils. Oxidative stress markers indicated by GSH and GSSG decreased linearly with increasing phenolic content.	[25]

Table 1. Cont.

High phenolic concentration vs. moderate phenolic concentration vs. low phenolic concentration olive oil	12 healthy subjects	366 vs. 164 vs. 2.7 mg/kg	Acute dose, crossover	Plasma F2-isoprostanes and plasma oxLDL	All olive oils promoted postprandial oxidative stress indicated by increased F2-isoprostanes, however, the degree of LDL oxidation decreased as the phenolic content in administered oil increased.	[75]
High phenolic concentration vs. low phenolic-enriched breakfast	21 hypercholesterolemic subjects	400 vs. 80 mg/kg	Acute dose, crossover	Plasma LPO and plasma F2-isoprostanes	Decrease in LPO and F2-isoprostanes with intake of the phenol-enriched breakfast compared to low phenol-enriched breakfast.	[76]
High phenolic concentration vs. low phenolic concentration olive oil	22 mild dyslipidemic subjects	166 vs. 2 mg/kg	49 day, crossover	Plasma lipids, plasma TXB2, plasma antioxidant capacity and urinary F2-isoprostanes	Plasma TXB2 decreased with phenol-rich olive oil supplementation. Plasma antioxidant capacity increased after phenol-rich olive oil administration. No effect on urinary F2-isoprostanes and plasma lipids between phenol-rich and phenol-poor olive oil were observed.	[77]
High phenolic concentration vs. low phenolic concentration olive oil	43 coronary heart disease subjects	161 vs. 14.67 mg/kg	3 week, crossover	Plasma oxLDL, plasma LPO and whole blood GSH-Px	Decrease in oxLDL and LPO and increase in GSH-Px upon phenol-rich olive oil consumption.	[78]
High phenolic concentration vs. moderate phenolic concentration vs. low phenolic concentration olive oil	30 healthy subjects	150 vs. 68 vs. 0 mg/kg	3 week, crossover	Plasma lipids and oxLDL	Sustained consumption of phenol-rich olive oil was more effective in protecting LDL from oxidation and in raising HDL-C than olive oils with lesser quantities of phenolics.	[79]
High phenolic concentration vs. moderate phenolic concentration vs. low phenolic concentration olive oil	12 healthy subjects	486 vs. 133 vs. 10 mg/kg	4 day, crossover	Plasma lipids, plasma oxLDL, plasma GSH-Px and urinary 8-oxo-dG	Short-term consumption of phenol-rich olive oil decreased plasma oxLDL, urinary 8-oxo-dG, and increased plasma HDL-C and GSH-Px, in a dose-dependent manner with the increasing phenolic content of the olive oil administered.	[80]
High phenolic concentration vs. low phenolic concentration olive oil	25 healthy subjects	21.6 vs. 3.0 mg/kg	3 week, crossover	Plasma antioxidant capacity and oxLDL	Plasma antioxidant capacity and oxLDL did not differ significantly between the phenol-rich and phenol-poor olive oil.	[81]
High phenolic concentration vs. low phenolic concentration olive oil	46 health subjects	308 vs. 43 mg/kg	3 week, crossover	Plasma lipids, plasma oxLDL and plasma LPO	No effect on plasma lipids, oxLDL, and LPO were noted between the phenol-rich and phenol-poor olive oils.	[82]

Table 1. Cont.

Olive oil with different phenolic concentrations	6 healthy subjects	1950 vs. 1462.5 vs. 975 vs. 487.5 mg/kg	Acute dose, cross over	Urinary F2-isoprostanes	A dose-dependent decrease in urinary excretion of F2-isoprostanes was noted upon administration of phenol-rich olive oil.	[29]
High phenolic concentration vs. low phenolic concentration olive oil	14 healthy subjects	303 vs. 0.3 mg/kg	4 week, crossover	Plasma oxLDL and serum antioxidant capacity	Increase in plasma antioxidant capacity but no change in oxLDL.	[83]
High phenolic concentration vs. low phenolic concentration olive oil	24 peripheral vascular disease subjects	800 vs. 60 mg/kg	12 week, crossover	Plasma lipids and plasma oxLDL	A lower oxLDL was noted in subjects after administration of phenol-rich olive oil. No difference in plasma lipids was observed.	[84]

4. Olive Oil Phenolic Compounds and Their Beneficial Effect on Plasma Lipoproteins

Elevated levels of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) have been established as risk factors for atherosclerosis, which is the primary cause of cardiovascular disease (CVD). However, on the other hand, elevated high density lipoprotein cholesterol (HDL-C) levels are believed to have protective, anti-inflammatory properties [85,86]. Data from a controlled human study containing 200 healthy male subjects, found a decrease in TC to HDL-C ratio with increasing phenolic content of the virgin olive oil consumed. An increase in HDL-C was also noted with increasing phenolic concentration of the oil [25]. Consumption of phenol rich virgin olive oils have resulted in increases in circulating HDL-C ranging between 5.1–6.7% in two further human studies [79,80]. Additionally, an earlier study showed a significant decrease in LDL-C after one week of phenol rich virgin olive oil consumption [87]. There are three human studies that showed olive oil phenolic compounds had no effect on blood lipid composition [77,82,84]. These studies used people with mild dyslipidemia and peripheral vascular disease (as opposed to healthy subjects used in the other studies) in two of the three studies and in the third study the authors propose the three week study period may not have been long enough to observe a treatment effect. However, it should be noted that in one study, four days was ample time to see a change in HDL-C [80].

Studies involving animals have demonstrated that ingestion of phenol rich virgin olive oil led to improvements in blood lipid profile. A study involving rabbits demonstrated a reduction in circulating TC and an increase in HDL-C upon virgin olive oil consumption. Furthermore, studies in rats have found that the intake of phenol rich virgin olive oil decreases TC, LDL-C and triglyceride (TG) levels [88] and substantially increases HDL-C concentrations [89].

5. Olive Oil Phenolic Compounds and Their Beneficial Effect on Lipid Oxidation

LDL oxidation (oxLDL) is considered to be a major risk factor for the development of atherosclerosis and CVD [90]. Oxidation of LDL causes damage to the vascular wall, stimulating macrophage uptake and formation of foam cells, which in turn result in the formation of plaque within the arterial wall [78,82,91,92]. Both human and animal *in vivo* studies have shown that the level at which LDL oxidizes, decreases linearly with increasing phenolic concentration

[25,26,75,78–80,87,93,94]. Two further mechanistic studies, demonstrated that phenolic compounds are able to bind to LDL and the authors suggest that this may account for the increase in LDL resistance to oxidation [72,95]. Although it is important to note that there have been conflicting results from three short term studies suggesting that the phenolic content of the virgin olive oil does not play a role in the lowering of oxLDL [81,82,83]. In addition, *in vitro* studies have found that the phenolic compounds extracted from virgin olive oil inhibit the oxidation of LDL-C [40,96–98].

6. Olive Oil Phenolic Compounds and Their Beneficial Effect on Oxidative DNA Damage

Oxidative damage to DNA is a precursor for human carcinogenesis [99] and it is well known that oxygen radicals continually attack human cells [74]. Unless damage to these cells is counteracted, DNA damage may result, and such damage can lead to cancer development [74]. A randomized cross-over intervention trial has shown that intake of phenol rich virgin olive oil decreases oxidative DNA damage by up to 30% compared to a low phenol virgin olive oil [74]. An additional study also demonstrated that after consumption of phenol rich virgin olive oil there was decreased urinary excretion of 8-oxo-deoxyguanosine (8oxodG) a systemic marker of DNA oxidation [99,100].

In support of these findings, animal studies have also reported that a diet enriched with olive oil phenolic compounds has a protective effect against DNA damage [101,102]. In agreement with these findings, a recent *in vitro* study reported that olive oil phenolic compounds showed DNA oxidation preventative activity [103].

7. Olive Oil Phenolic Compounds and Their Beneficial Effect on Additional Markers of Oxidation

Oxidative stress produced by reactive oxygen species (ROS) has been linked to a number of diseases such as atherosclerosis, certain cancers and neurodegenerative diseases [90,104] and is considered as a by-product of aerobic metabolism. Goya *et al.* [105] treated a sample of human hepatoma HepG2 cells with hydroxytyrosol and found that there was a decrease in ROS production. Similarly olive oil phenolic compounds have been shown to scavenge ROS under natural and chemically simulated oxidative stress conditions [106–108]. A further study by Moreno *et al.* [109] demonstrated that tyrosol modulated ROS production, in murine macrophages [109].

Total plasma antioxidant activity has also been reported to increase in humans after the ingestion of olive oil phenolic compounds [73,74,77]. However, one study did not report an increase in the total blood antioxidant capacity [81].

Oxidative stress can be indicated by the presence of markers such as F₂-isoprostanes, lipid peroxides (LPO), oxidized glutathione (GSSG), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px). F₂-isoprostanes are a result of the free radical induced peroxidation of arachidonic acid. LPO is more than likely a by-product of the oxidation of fatty acids [76] and depletion of the protective GSH precedes lipid oxidation and atherogenesis *in vivo* [110].

Human studies have shown beneficial effects of olive oil phenolic compounds on these aforementioned markers of oxidative stress. A randomized cross over study found the intake of a olive oil phenolic-enriched breakfast significantly lowered F₂-isoprostane levels compared to a low phenolic-enriched breakfast [76]. Visioli and colleagues [29] demonstrated that consumption of a

phenolic-rich virgin olive oil was associated with a significant decrease in urinary excretion of F₂-isoprostanes. Another human study did not find an effect of olive oil phenolics on F₂-isoprostane levels, this may be possibly due to the considerably low phenolic content of the oil administered (166 mg/kg) [77] compared to a phenolic content up 1950 mg/kg in the Visioli *et al.* study [29]. An animal study also found that administration of hydroxytyrosol-containing olive oil waste water to rats exposed to cigarette smoke, lowered F₂-isoprostane levels significantly ($p < 0.05$) [64].

Covas and colleagues [25] found that phenol-rich virgin olive oil beneficially modulated the balance between GSH and GSSG, while Weinbrenner and colleagues [80] found an increase in GSH-Px after phenol-rich virgin olive oil administration in human subjects. Moreover, a decrease in LPO after olive oil phenolic administration has been noted. More recently, olive oil phenolic compounds sourced from olive mill waste water were found to increase GSH concentrations in human blood [111]. Two further studies have also shown that olive oil phenolic compounds reduce oxidative damage to both red blood cells and renal cells [108,112].

8. Olive Oil Phenolic Compounds and Their Beneficial Effect on Markers of Inflammation

Elevated concentrations of inflammation markers in serum are associated with increased cardiovascular risk [113]. Plasma thromboxane B₂ (TXB₂) and leukotriene B₄ (LTB₄) are known pro-inflammatory agents. TXB₂ has the ability to increase blood platelet aggregation and LTB₄ has a chemostatic effect on neutrophils, directing the cells to damaged tissue [73,114]. These inflammatory agents are known to produce the pain, redness and swelling associated with inflammation [115]. Bogani and colleagues [73] found a decrease in TXB₂ and LTB₄ concentrations with increasing phenolic concentration of the olive oil. These results were also in accordance with previous investigations [77,80,116].

The inflammatory markers, Interleukin-6 (IL-6) and C-reactive protein (CRP) have been shown to be predictors for CVD also [70]. IL-6 is a pro-inflammatory agent that stimulates inflammation in response to trauma and CRP generally rises when inflammation is present [70]. Fito and colleagues [70] found that consumption of olive oil phenolic compounds from a daily dose of virgin olive oil decreased the circulating concentrations of both IL-6 and CRP in 28 stable coronary heart patients.

In vitro study findings also support the anti-inflammatory capacity of olive oil phenolic compounds. Olive oil phenolics have been found to decrease arachidonic acid release and arachidonic acid metabolite synthesis *in vitro* and both of these are involved in the inflammatory process [109]. The olive oil phenolic compound, oleocanthal was shown to inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) activity (both involved in the inflammatory process) in the same way as the anti-inflammatory drug, ibuprofen does [117]. Inhibition of COX enzymes results in the reduction of arachidonate to the eicosanoids, prostaglandins and thromboxane, in the inflammatory pathway [114,118].

9. Olive Oil Phenolic Compounds and Their Beneficial Effect on Platelet Function

Blood platelets have been demonstrated to play a role in CVD and atherosclerosis development. Continual damage to the vascular epithelium results in the development of lesions and these lesions stimulate endothelial adhesion molecule expression, platelet activity and aggregation [119,120].

Circulating monocytes are attracted by these particular molecules and adhere to the endothelium and differentiate into macrophages, which in turn scavenge LDL and triglyceride (TG) rich lipoproteins, becoming foam cells and forming fatty streaks [121].

Olive oil phenolic compounds have been shown to inhibit endothelial adhesion molecule expression upon incubation with human umbilical vein endothelial cells [27]. They also have been demonstrated to inhibit human platelet activity *in vitro* [122]. The phenolic compound, hydroxytyrosol has been noted to completely inhibit platelet aggregation in human blood (*in vitro*) in the range of 100–400 μM [123]. A more recent investigation, demonstrated that a number of olive oil phenolic compounds (such as oleuropein aglycone and luteolin) also were potent inhibitors of platelet aggregation [124]. Virgin olive oil containing a high content (400 mg/kg) of phenolic compounds has been demonstrated to also decrease plasminogen activator inhibitor-1 (PAI-1) and factor VII (FVII). Both PAI-1 and FVII are pro-coagulant factors that have been linked to the development of coronary heart disease (CHD) [71]. Furthermore, olive oil phenolics have been shown to decrease homocysteine, which has been linked to increased adhesiveness of the endothelium [125].

10. Olive Oil Phenolic Compounds and Their Beneficial Effect on Cellular Function

Cell proliferation and suppressed cell death are underlying factors for tumor formation and progression [126]. Research to date has shown that the olive oil phenolic, hydroxytyrosol inhibits cell proliferation in human promyelocytic HL60 leukemia cells and in human colon cancer lines [127–129]. Hashim and colleagues [130] demonstrated a dose-related inhibition of colon cancer cell invasion by olive oil phenolic compounds. Also, hydroxytyrosol has been found to exert strong anti-proliferative effects against human colon adenocarcinoma cells [131]. More recently, research using MCF-7 and SKBR3 breast cancer cells showed that phenolic compounds inhibit cell growth in these cell lines in a dose dependent manner and reduce expression of the HER2 oncogene which plays a integral role in malignant transformation, tumorigenesis, and metastasis [132–134]. Furthermore, oleuropein and hydroxytyrosol have been show to induce cell death of MCF-7 human breast cancer cells [135]. Olive oil phenolics have also been found to improve cell integrity and viability in CaCo2 cells [128,136].

The olive oil phenolic compound, oleocanthal has been implicated in the reduced incidence of Alzheimer's disease in Mediterranean populations via two mechanisms. First, in Alzheimer's disease a microtubule-associated protein (Tau) involved in the promotion of microtubule assembly and stability begin to aggregate into neurofibrillary tangles. Li and colleagues [137] demonstrated that oleocanthal inhibits tau aggregation. Second, beta-amyloid ($A\beta$) oligomers (also referred to as ADDLs) have also been suggested to be involved in the development of Alzheimer's disease. These ADDLs are believed to bind to postsynaptic sites and cause synaptic and neuronal loss. Pitt and colleagues [138] have demonstrated that oleocanthal has the capacity to alter the oligomerization state of ADDLs whilst protecting neurons from the synaptopathological effects of ADDLs. Thus, oleocanthal protects neurons from ADDL induced synaptic deterioration and additionally promotes the antibody clearance of ADDLs [138]. A further study demonstrated the neuro-protection of hydroxytyrosol in rat brains. In this study it was found that hydroxytyrosol reduced lactate dehydrogenase activity which has been closely related with a reduction in brain lipid peroxidation [139].

11. Olive Oil Phenolic Compounds and Their Beneficial Effect on Microbial Activity

In vitro research has shown that olive oil phenolic compounds have antimicrobial properties. Particularly, the phenolic compounds, oleuropein, hydroxytyrosol and tyrosol have demonstrated potent antimicrobial activity against several strains of bacteria responsible for intestinal and respiratory infections [140]. Romero and colleagues [141] found that the dialdehydic form of decarboxymethyl ligstroside is not hydrolyzed in the stomach and therefore aids in inhibiting the growth of *Helicobacter pylori* bacteria. *Helicobacter pylori* bacteria are linked to the development of peptic ulcers and some types of gastric cancer. Hydroxytyrosol and oleuropein have also been shown to be cytotoxic to a large number of bacterial strains [142].

12. Olive Oil Phenolic Compounds and Their Beneficial Effect on Bone

One study to date has investigated the effect of olive oil phenolic compounds on bone [143]. In this study, both tyrosol and hydroxytyrosol increased bone formation in rats significantly. Further studies are now required to substantiate these findings.

13. Conclusions

In conclusion, olive oil phenolic compounds are highly bioavailable in humans. The high bioavailability of such compounds lends support to evidence that these phenolic components exert beneficial effects on health. Although, the beneficial health effects of virgin olive oil ingestion are well known, it is only recent that the biological properties of olive oil phenolic compounds have been investigated. In experimental studies (*in vivo* and *in vitro*), olive oil phenolic compounds have been shown to beneficially alter lipid composition, platelet and cellular function, microbial activity and bone formation, as well as reduce oxidative damage and inflammation. The modes of action detailed in the paper, may explain the low rate of diet-related diseases amongst populations residing in the Mediterranean region. For example, the anti-atherogenic effects associated with the ingestion of virgin olive oil may explain the low rate of cardiovascular disease in Mediterranean populations. Since DNA oxidative damage is a mechanism underlying cancer development, the protective effects of olive oil phenolic compounds may explain some of the differences in cancer incidence between Mediterranean populations and other populations in the world. The anti-inflammatory effects that arise from the ingestion of olive oil phenolic compounds have been shown to provide protection against diseases marked by an inflammatory component. This may, along with other modes of action, partly explain the low rate of CVD mortality and certain types of cancer in populations residing in the Mediterranean. Olive oil phenolic compounds may also be useful in the treatment of some infectious diseases. Finally, although more studies are required, findings demonstrating the beneficial effects of olive oil phenolics in relation to bone health, may aid in partly explaining the low incidence of osteoporosis in populations residing in the Mediterranean area. These biological properties may have a significant impact on population health through the reduction in incidence of chronic degenerative disease development.

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References

1. De Lorgeril, M.; Salen, P.; Martin, J.L.; Monjaud, I.; Delaye, J.; Mamelle, N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* **1999**, *99*, 779–785.
2. Singh, R.B.; Dubnov, G.; Niaz, M.A.; Ghosh, S.; Singh, R.; Rastogi, S.S.; Manor, O.; Pella, D.; Berry, E.M. Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): A randomised single-blind trial. *Lancet* **2002**, *360*, 1455–1461.
3. Stark, A.H.; Madar, Z. Olive oil as a functional food: epidemiology and nutritional approaches. *Nutr. Rev.* **2002**, *60*, 170–176.
4. Trichopoulou, A.; Costacou, T.; Bamia, C.; Trichopoulos, D. Adherence to a Mediterranean diet and survival in a Greek population. *N. Engl. J. Med.* **2003**, *348*, 2599–2608.
5. Visioli, F.; Galli, C. Natural antioxidants and prevention of coronary heart disease: the potential role of olive oil and its minor constituents. *Nutr. Metab. Cardio. Dis.* **1995**, *5*, 306–314.
6. Fortes, C.; Forastiere, F.; Farchi, S.; Mallone, S.; Trequattrinni, T.; Anatra, F.; Schmid, G.; Perucci, C.A. The protective effect of the Mediterranean diet on lung cancer. *Nutr. Cancer* **2003**, *46*, 30–37.
7. Grasso, S.; Siracusa, L.; Spatafora, C.; Renis, M.; Tringali, C. Hydroxytyrosol lipophilic analogues: Enzymatic synthesis, radical scavenging activity and DNA oxidative damage protection. *Bioorg. Chem.* **2006**, *35*, 137–152.
8. Herrera, M.D.; Perez-Guerrero, C.; Marhuenda, E.; Ruiz-Gutierrez, V. Effects of dietary oleic-rich oils (virgin olive and high-oleic-acid sunflower) on vascular reactivity in Wistar-Kyoto and spontaneously hypertensive rats. *Br. J. Nutr.* **2001**, *86*, 349–357.
9. Matalas, A.-L.; Zampelas, A.; Stavrinos, V.; Wolinsky, I. *The Mediterranean Diet: Constituents and Health Promotion*; CRC Press: Boca Raton, FL, USA, 2001.
10. Galeone, C.; Talamini, R.; Levi, F.; Pelucchi, C.; Negri, E.; Giacosa, A.; Montella, M.; Franceschi, S.; La Vecchia, C. Fried foods, olive oil and colorectal cancer. *Ann. Oncol.* **2006**, *18*, 36–39.
11. Filik, L.; Ozyilkan, O. Olive-oil consumption and cancer risk. *Eur. J. Clin. Nutr.* **2003**, *57*, 191.
12. Hu, F.B. The Mediterranean diet and mortality—olive oil and beyond. *N. Engl. J. Med.* **2003**, *348*, 2595–2596.
13. Kok, F.J.; Kromhout, D. Atherosclerosis—epidemiological studies on the health effects of a Mediterranean diet. *Eur. J. Nutr.* **2004**, *43*, 1S–5S.
14. Kris-Etherton, P.; Eckel, R.H.; Howard, B.V.; St. Jeor, S.; Bazzarre, T.L. AHA Science advisory: Lyon diet heart study. benefits of a mediterranean-style, national cholesterol education program/american heart association step i dietary pattern on cardiovascular disease. *Circulation* **2001**, *103*, 1823–1825.

15. Kushi, L.H.; Lenart, E.B.; Willett, W.C. Health implications of Mediterranean diets in light of contemporary knowledge. 2. Meat, wine, fats, and oils. *Am. J. Clin. Nutr.* **1995**, *61*, 1416S–1427S.
16. Willett, W.C.; Sacks, F.; Trichopoulou, A.; Drescher, G.; Ferro-Luzzi, A.; Helsing, E.; Trichopoulos, D. Mediterranean diet pyramid: a cultural model for healthy eating. *Am. J. Clin. Nutr.* **1995**, *61*, 1402S–1406S.
17. Fung, T.T.; Rexrode, K.M.; Mantzoros, C.S.; Manson, J.E.; Willett, W.C.; Hu, F.B. Mediterranean diet and incidence of and mortality from coronary heart disease and stroke in women. *Circulation* **2009**, *119*, 1093–1100.
18. Corona, G.; Spencer, J.P.E.; Dessi, M.A. Extra virgin olive oil phenolics: absorption, metabolism, and biological activities in the GI tract. *Toxicol. Ind. Health* **2009**, *25*, 285–293.
19. Tripoli, E.; Giammanco, M.; Tabacchi, G.; Di Majo, D.; Giammanco, S.; La Guardia, M. The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr. Res. Rev.* **2005**, *18*, 98–112.
20. Harper, C.R.; Edwards, M.C.; Jacobson, T.A. Flaxseed oil supplementation does not affect plasma lipoprotein concentration or particle size in human subjects. *J. Nutr.* **2006**, *136*, 2844–2848.
21. Aguilera, C.M.; Mesa, M.D.; Ramirez-Tortosa, M.C.; Nestares, M.T.; Ros, E.; Gil, A. Sunflower oil does not protect against LDL oxidation as virgin olive oil does in patients with peripheral vascular disease. *Clin. Nutr.* **2004**, *23*, 673–681.
22. Visioli, F.; Galli, C. Biological properties of olive oil phytochemicals. *Crit. Rev. Food Sci. Nutr.* **2002**, *42*, 209–221.
23. Psaltopoulou, T.; Naska, A.; Orfanos, P.; Trichopoulos, D.; Mountokalakis, T.; Trichopoulou, A. Olive oil, the Mediterranean diet, and arterial blood pressure: the Greek European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Am. J. Clin. Nutr.* **2004**, *80*, 1012–1018.
24. Martin-Moreno, J.M.; Willett, W.C.; Gorgojo, L.; Banegas, J.R.; Rodriguez-Artalejo, F.; Fernandez-Rodriguez, J.C.; Maisonneuve, P.; Boyle, P. Dietary fat, olive oil intake and breast cancer risk. *Int. J. Cancer* **1994**, *58*, 774–780.
25. Covas, M.I.; Nyyssonen, K.; Poulsen, H.E.; Kaikkonen, J.; Zunft, H.J.; Kiesewetter, H.; Gaddi, A.; de la Torre, R.; Mursu, J.; Baumler, H.; Nascetti, S.; Salonen, J.T.; Fito, M.; Virtanen, J.; Marrugat, J.; Group, E.S. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann. Int. Med.* **2006**, *145*, 333–341.
26. Nicolaiew, N.; Lemort, N.; Adorni, L.; Berra, B.; Montorfano, G.; Rapelli, S.; Cortesi, N.; Jacotot, B. Comparison between extra virgin olive oil and oleic acid rich sunflower oil: effects on postprandial lipemia and LDL susceptibility to oxidation. *Ann. Nutr. Metab.* **1998**, *42*, 251–260.
27. Carluccio, M.A.; Siculella, L.; Ancora, M.A.; Massaro, M.; Scoditti, E.; Storelli, C.; Visioli, F.; Distanti, A.; De Caterina, R. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. *Arterio. Throm. Vasc. Bio.* **2003**, *23*, 622–629.
28. Tuck, K.L.; Hayball, P.J. Major phenolic compounds in olive oil: metabolism and health effects. *J. Nutr. Biochem.* **2002**, *13*, 636–644.

29. Visioli, F.; Caruso, D.; Galli, C.; Viappiani, S.; Galli, G.; Sala, A. Olive oils rich in natural catecholic phenols decrease isoprostane excretion in humans. *Biochem. Biophys. Res. Comm.* **2000**, *278*, 797–799.
30. Visioli, F.; Galli, C.; Bornet, F.; Mattei, A.; Patelli, R.; Galli, G.; Caruso, D. Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Lett.* **2000**, *468*, 159–160.
31. Bai, C.; Yan, X.; Takenaka, M.; Sekiya, K.; Nagata, T. Determination of synthetic hydroxytyrosol in rat plasma by GC-MS. *J. Agric. Food Chem.* **1998**, *46*, 3998–4001.
32. Vissers, M.N.; Zock, P.L.; Roodenburg, A.J.; Leenen, R.; Katan, M.B. Olive oil phenols are absorbed in humans. *J. Nutr.* **2002**, *132*, 409–417.
33. Miro Casas, E.; Albadalejo, M. F.; Covas Planells, M. I.; Colomer, F. M.; Lamuela Raventos, R. M.; de la Torre Fornell, R. Tyrosol bioavailability in humans after ingestion of virgin olive oil. *Clin. Chem.* **2001**, *47*, 341–343.
34. Bruni, N.; Cortesi, N.; Fiorino, P. Influence of agricultural techniques, cultivar and area of origin on characteristics of virgin olive oil and levels of some of its "minor" components. *Olivae* **1994**, *1*, 28–34.
35. Esti, M.; Cinquanta, L.; La Notte, E. Phenolic compounds in different olive varieties. *J. Agric. Food Chem.* **1998**, *46*, 32–35.
36. Gomez-Alonzo, S.; Salvador, M.D.; Fregapane, G. Phenolic compounds profile of Cornicarba virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 6812–6817.
37. Vinha, A.F.; Ferreres, F.; Silva, B.M.; Valentao, P.; Goncalves, A.; Pereira, J.A.; Oliveira, M.B.; Seabra, R.M.; Andrade, P.B. Phenolic profiles of Portuguese olive fruits (*Olea europaea* L.): Influences of cultivar and geographical origin. *Food Chem.* **2005**, *89*, 561–568.
38. Cerretani, L.; Bendini, A.; Rotondi, A.; Lercker, G.; Toschi, T.G. Analytical comparison of monovarietal virgin olive oils obtained by both a continuous industrial plant and low-scale mill. *Eur. J. Lipid Sci. Technol.* **2005**, *107*, 93–100.
39. Sivakumar, G.; Bati, C.B.; Uccella, N. HPLC-MS screening of the antioxidant profile of Italian olive cultivars. *Chem. Nat. Comp.* **2005**, *41*, 588–591.
40. Franconi, F.; Coinu, R.; Carta, S.; Urgeghe, P.P.; Ieri, F.; Mulinacci, N.; Romani, A. Antioxidant effect of two virgin olive oils depends on the concentration and composition of minor polar compounds. *J. Agric. Food Chem.* **2006**, *54*, 3121–3125.
41. Carrasco Pancorbo, A.; Cruces-Blanco, C.; Segura Carretero, A.; Fernandez Gutierrez, A. Sensitive determination of phenolic acids in extra-virgin olive oil by capillary zone electrophoresis. *J. Agric. Food Chem.* **2004**, *52*, 6687–6693.
42. Caravaca, A.M.G.; Pancorbo, A.C.; Diaz, B.C.; Carretero, A.S.; Gutierrez, A.F. Electrophoretic identification and quantification of compounds in the polyphenolic fraction of extra-virgin olive oil. *Electrophoresis* **2005**, *26*, 3538–3551.
43. Romero, M.P.; Tovar, M.J.; Girona, J.; Motilva, M.J. Changes in the HPLC phenolic profile of virgin olive oil from young trees (*Olea europaea* L. Cv. Arbequina) grown under different deficit irrigation strategies. *J. Agric. Food Chem.* **2002**, *50*, 5349–5354.
44. Gomez-Rico, A.; Salvador, M.D.; La Greca, M.; Fregapane, G. Phenolic and volatile compounds of extra virgin olive oil (*Olea europaea* L. Cv. Cornicabra) with regard to fruit ripening and irrigation management. *J. Agric. Food Chem.* **2006**, *54*, 7130–7136.

45. Gimeno, E.; Castellote, A.I.; Lamuela-Raventos, R.M.; De la Torre, M.C.; Lopez-Sabater, M.C. The effects of harvest and extraction methods on the antioxidant content (phenolics, alpha-tocopherol, and beta-carotene) in virgin olive oil. *Food Chem.* **2002**, *78*, 207–211.
46. Brenes, M.; Garcia, A.; Garcia, P.; Rios, J.J.; Garrido, A. Phenolic compounds in Spanish olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535–3540.
47. Kalua, C.M.; Allen, M.S.; Bedgood, D.R., Jr.; Bishop, A.G.; Prenzler, P.D. Discrimination of olive oils and fruits into cultivars and maturity stages based on phenolic and volatile compounds. *J. Agric. Food Chem.* **2005**, *53*, 8054–8062.
48. Tovar, M.J.; Motilva, M.J.; Romero, M.P. Changes in the phenolic composition of virgin olive oil from young trees (*Olea europaea* L. cv. Arbequina) grown under linear irrigation strategies. *J. Agric. Food Chem.* **2001**, *49*, 5502–5508.
49. Ranalli, A.; Ferrante, M.L.; De Mattia, G.; Costantini, N. Analytical evaluation of virgin olive oil of first and second extraction. *J. Agric. Food Chem.* **1999**, *47*, 417–424.
50. Kalua, C.M.; Bedgood, D.R., Jr.; Bishop, A.G.; Prenzler, P.D. Changes in volatile and phenolic compounds with malaxation time and temperature during virgin olive oil production. *J. Agric. Food Chem.* **2006**, *54*, 7641–7651.
51. Mailer, R.J.; Ayton, J. Comparison of olive oil (*Olea europaea*) quality extracted by stonemill and hammermill. *NZ. J. Crop Hort. Sci.* **2004**, *32*, 325–330.
52. Fregapane, G.; Lavelli, V.; Leon, S.; Kapuralin, J.; Desamparados Salvador, M. Effect of filtration on virgin olive oil stability during storage. *Eur. J. Lip. Sci. Technol.* **2006**, *108*, 134–142.
53. Di Giovacchino, L.; Sestili, S.; Di Vincenzo, D. Influence of olive processing on virgin olive oil quality. *Eur. J. Lip. Sci. Technol.* **2002**, *104*, 587–601.
54. Brenes, M.; Garcia, A.; Garcia, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609–5614.
55. Gutierrez, F.; Fernandez, J.L. Determinant parameters and components in the storage of virgin olive oil. Prediction of storage time beyond which the oil is no longer of "extra" quality. *J. Agric. Food Chem.* **2002**, *50*, 571–577.
56. Okogeri, O.; Tasioula-Margari, M. Changes occurring in phenolic compounds and alpha-tocopherol of virgin olive oil during storage. *J. Agric. Food Chem.* **2002**, *50*, 1077–1080.
57. Rastrelli, L.; Passi, S.; Ippolito, F.; Vacca, G.; De Simone, F. Rate of degradation of alpha-tocopherol, squalene, phenolics, and polyunsaturated fatty acids in olive oil during different storage conditions. *J. Agric. Food Chem.* **2002**, *50*, 5566–5570.
58. Brenes, M.; Garcia, A.; Dobarganes, M.C.; Velasco, J.; Romero, C. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 5962–5967.
59. Gomez-Alonso, S.; Fregapane, G.; Salvador, M.D.; Gordon, M.H. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *J. Agric. Food Chem.* **2003**, *51*, 667–672.
60. Cicerale, S.; Conlan, X.A.; Barnett, N.W.; Sinclair, A.J.; Keast, R.S. Influence of heat on biological activity and concentration of oleocanthal—A natural anti-inflammatory agent in virgin olive oil. *J. Agric. Food Chem.* **2009**, *57*, 1326–1330.

61. Carrasco-Pancorbo, A.; Cerretani, L.; Bendini, A.; Segura-Carretero, A.; Gallina-Toschi, T.; Fernandez-Gutierrez, A. Analytical determination of polyphenols in olive oils. *J. Sep. Sci.* **2005**, *28*, 837–858.
62. Cicerale, S.; Conlan, X.A.; Sinclair, A.J.; Keast, R.S.J. Chemistry and health of olive oil phenolics. *Crit. Rev. Food Sci. Nutr.* **2009**, *49*, 218–236.
63. Martini, F.H. *Fundamentals of Anatomy and Physiology*, 7 ed.; Pearson Education Inc.: San Francisco, CA, USA, 2006.
64. Visioli, F.; Galli, C.; Plasmati, E.; Viappiani, S.; Hernandez, A.; Colombo, C.; Sala, A. Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress. *Circulation* **2000**, *102*, 2169–2171.
65. Caruso, D.; Visioli, F.; Patelli, R.; Galli, C.; Galli, G. Urinary excretion of olive oil phenols and their metabolites in humans. *Metabolism* **2001**, *50*, 1426–1428.
66. Tuck, K.L.; Freeman, M.P.; Hayball, P.J.; Stretch, G.L.; Stupans, I. The *in vivo* fate of hydroxytyrosol and tyrosol, antioxidant phenolic constituents of olive oil, after intravenous and oral dosing of labeled compounds to rats. *J. Nutr.* **2001**, *131*, 1993–1996.
67. Edgecombe, S.; Stretch, G.L.; Hayball, P.J. Oleuropein, an antioxidant polyphenol from olive oil, is poorly absorbed from isolated perfused rat intestine. *J. Nutr.* **2000**, *130*, 2996–3002.
68. Manna, C.; Galletti, P.; Maisto, G.; Cucciolla, V.; D'Angelo, S.; Zappia, V. Transport mechanism and metabolites of olive oil hydroxytyrosol in Caco-2 cells. *FEBS Letters* **2000**, *470*, 341–344.
69. Visioli, F.; Caruso, D.; Plasmati, E.; Patelli, R.; Mulinacci, N.; Romani, A.; Galli, G.; Galli, C. Hydroxytyrosol, as a component of olive mill waste water, is dose- dependently absorbed and increases the antioxidant capacity of rat plasma. *Free Rad. Res.* **2001**, *34*, 301–305.
70. Fito, M.; Cladellas, M.; de la Torre, R.; Marti, J.; Munoz, D.; Schroder, H.; Alcantara, M.; Pujadas-Bastardes, M.; Marrugat, J.; Lopez-Sabater, M.C.; Bruguera, J.; Covas, M.I. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. *Eur. J. Clin. Nutr.* **2008**, *62*, 570–574.
71. Ruano, J.; Lopez-Miranda, J.; de la Torre, R.; Delgado-Lista, J.; Fernandez, J.; Caballero, J.; Covas, M.I.; Jimenez, Y.; Perez-Martinez, P.; Marin, C.; Fuentes, F.; Perez-Jimenez, F. Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. *Am. J. Clin. Nutr.* **2007**, *86*, 341–346.
72. Gimeno, E.; de la Torre-Carbot, K.; Lamuela-Raventos, R.M.; Castellote, A.I.; Fito, M.; de la Torre, R.; Covas, M.I.; Carmen Lopez-Sabater, M. Changes in the phenolic content of low density lipoprotein after olive oil consumption in men. A randomized crossover controlled trial. *Br. J. Nutr.* **2007**, 1–8.
73. Bogani, P.; Galli, C.; Villa, M.; Visioli, F. Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis* **2007**, *190*, 181–186.
74. Salvini, S.; Sera, F.; Caruso, D.; Giovannelli, L.; Visioli, F.; Saieva, C.; Masala, G.; Ceroti, M.; Giovacchini, V.; Pitozzi, V.; Galli, C.; Romani, A.; Mulinacci, N.; Bortolomeazzi, R.; Dolara, P.; Palli, D. Daily consumption of a high-phenol extra-virgin olive oil reduces oxidative DNA damage in postmenopausal women. *Br. J. Nutr.* **2006**, *95*, 742–751.
75. Covas, M.I.; de la Torre, K.; Farre-Albaladejo, M.; Kaikkonen, J.; Fito, M.; Lopez-Sabater, C.; Pujadas-Bastardes, M.A.; Joglar, J.; Weinbrenner, T.; Lamuela-Raventos, R.M.; de la Torre, R.

- Postprandial LDL phenolic content and LDL oxidation are modulated by olive oil phenolic compounds in humans. *Free Rad. Biol. Med.* **2006**, *40*, 608–616.
76. Ruano, J.; Lopez-Miranda, J.; Fuentes, F.; Moreno, J.A.; Bellido, C.; Perez-Martinez, P.; Lozano, A.; Gomez, P.; Jimenez, Y.; Perez Jimenez, F. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *J. Am. Coll. Cardiol.* **2005**, *46*, 1864–1868.
 77. Visioli, F.; Caruso, D.; Grande, S.; Bosisio, R.; Villa, M.; Galli, G.; Sirtori, C.; Galli, C. Virgin Olive Oil Study (VOLOS): Vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *Eur. J. Nutr.* **2005**, *44*, 121–127.
 78. Fito, M.; Cladellas, M.; de la Torre, R.; Marti, J.; Alcantara, M.; Pujadas-Bastardes, M.; Marrugat, J.; Bruguera, J.; Lopez-Sabater, M.C.; Vila, J.; Covas, M.I. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. *Atherosclerosis* **2005**, *181*, 149–158.
 79. Marrugat, J.; Covas, M.I.; Fito, M.; Schroder, H.; Miro-Casas, E.; Gimeno, E.; Lopez-Sabater, M.C.; de la Torre, R.; Farre, M. Effects of differing phenolic content in dietary olive oils on lipids and LDL oxidation--a randomized controlled trial. *Eur. J. Nutr.* **2004**, *43*, 140–147.
 80. Weinbrenner, T.; Fito, M.; de la Torre, R.; Saez, G.T.; Rijken, P.; Tormos, C.; Coolen, S.; Albaladejo, M.F.; Abanades, S.; Schroder, H.; Marrugat, J.; Covas, M.I. Olive oils high in phenolic compounds modulate oxidative/antioxidative status in men. *J. Nutr.* **2004**, *134*, 2314–2321.
 81. Moschandreas, J.; Vissers, M.N.; Wiseman, S.; van Putte, K.P.; Kafatos, A. Extra virgin olive oil phenols and markers of oxidation in Greek smokers: a randomized cross-over study. *Eur. J. Clin. Nutr.* **2002**, *56*, 1024–1029.
 82. Vissers, M.N.; Zock, P.L.; Wiseman, S.A.; Meyboom, S.; Katan, M B. Effect of phenol-rich extra virgin olive oil on markers of oxidation in healthy volunteers. *Eur. J. Clin. Nutr.* **2001**, *55*, 334–341.
 83. Bonanome, A.; Pagnan, A.; Caruso, D.; Toia, A.; Xamin, A.; Fedeli, E.; Berra, B.; Zamburlini, A.; Ursini, F.; Galli, G. Evidence of postprandial absorption of olive oil phenols in humans. *Nutr. Metab. Cardiovas. Dis.* **2000**, *10*, 111–120.
 84. Ramirez-Tortosa, M.C.; Urbano, G.; Lopez-Jurado, M.; Nestares, T.; Gomez, M.C.; Mir, A.; Ros, E.; Mataix, J.; Gil, A. Extra-virgin olive oil increases the resistance of LDL to oxidation more than refined olive oil in free-living men with peripheral vascular disease. *J. Nutr.* **1999**, *129*, 2177–2183.
 85. Gordon, T.; Kannel, W.B.; Castelli, W.P.; Dawber, T.R. Lipoproteins, cardiovascular disease, and death. The Framingham study. *Arch. Intern. Med.* **1981**, *141*, 1128–1131.
 86. Chrysohoou, C.; Pitsavos, C.; Skoumas, J.; Masoura, C.; Katinioti, A.; Panagiotakos, D.; Stefanadis, C. The emerging anti-inflammatory role of HDL-cholesterol, illustrated in cardiovascular disease free population; the ATTICA study. *Int. J. Cardio.* **2006**, *122*, 29–33.
 87. Gimeno, E.; Fito, M.; Lamuela-Raventos, R.M.; Castellote, A.I.; Covas, M.; Farre, M.; de La Torre-Boronat, M.C.; Lopez-Sabater, M.C. Effect of ingestion of virgin olive oil on human low-density lipoprotein composition. *Eur. J. Clin. Nutr.* **2002**, *56*, 114–120.

88. Gorinstein, S.; Leontowicz, H.; Lojek, A.; Leontowicz, M.; Ciz, M.; Krzeminski, R.; Gralak, M.; Czerwinski, J.; Jastrzebski, Z.; Trakhtenberg, S.; Grigelmo-Miguel, N.; Soliva-Fortuny, R.; Martin-Belloso, O. Olive oils improve lipid metabolism and increase antioxidant potential in rats fed diets containing cholesterol. *J. Agric. Food Chem.* **2002**, *50*, 6102–6108.
89. Mangas-Cruz, M.A.; Fernandez-Moyano, A.; Albi, T.; Guinda, A.; Relimpio, F.; Lanzon, A.; Pereira, J.L.; Serrera, J.L.; Montilla, C.; Astorga, R.; Garcia-Luna, P.P. Effects of minor constituents (non-glyceride compounds) of virgin olive oil on plasma lipid concentrations in male Wistar rats. *Clin. Nutr.* **2001**, *20*, 211–215.
90. Witztum, J.L. The oxidation hypothesis of atherosclerosis. *Lancet* **1994**, *344*, 793–795.
91. Coni, E.; Di Benedetto, R.; Di Pasquale, M.; Masella, R.; Modesti, D.; Mattei, R.; Carlini, E.A. Protective effect of oleuropein, an olive oil biophenol, on low density lipoprotein oxidizability in rabbits. *Lipids* **2000**, *35*, 45–54.
92. Patrick, L.; Uzick, M. Cardiovascular disease: C-reactive protein and the inflammatory disease paradigm: HMG-CoA reductase inhibitors, alpha-tocopherol, red yeast rice, and olive oil polyphenols. A review of the literature. *Altern. Med. Rev.* **2001**, *6*, 248–271.
93. Ramirez-Tortosa, C.; Lopez-Pedrosa, J.M.; Suarez, A.; Ros, E.; Mataix, J.; Gil, A. Olive oil- and fish oil-enriched diets modify plasma lipids and susceptibility of LDL to oxidative modification in free-living male patients with peripheral vascular disease: the Spanish Nutrition Study. *Br. J. Nutr.* **1999**, *82*, 31–39.
94. Ochoa, J.J.; Quiles, J.L.; Ramirez-Tortosa, C.; Mataix, J.; Huertas, J.R. Dietary oil high in oleic acid but with different unsaponifiable fraction contents have different effects in fatty acid composition and peroxidation in rabbit LDL. *Nutrition* **2002**, *18*, 60–65.
95. De la Torre-Carbot, K.; Chavez-Servin, J.L.; Jauregui, O.; Castellote, A.I.; Lamuela-Raventos, R.M.; Fito, M.; Covas, M.I.; Munoz-Aguayo, D.; Lopez-Sabater, M.C. Presence of virgin olive oil phenolic metabolites in human low density lipoprotein fraction: determination by high-performance liquid chromatography-electrospray ionization tandem mass spectrometry. *Analyt. Chim. Acta* **2007**, *583*, 402–410.
96. Berrougui, H.; Cloutier, M.; Isabelle, M.; Khalil, A. Phenolic-extract from argan oil (*Argania spinosa* L.) inhibits human low-density lipoprotein (LDL) oxidation and enhances cholesterol efflux from human THP-1 macrophages. *Atherosclerosis* **2006**, *184*, 389–396.
97. Masella, R.; Vari, R.; D'Archivio, M.; Di Benedetto, R.; Matarrese, P.; Malorni, W.; Scaccocchio, B.; Giovannini, C. Extra virgin olive oil biophenols inhibit cell-mediated oxidation of LDL by increasing the mRNA transcription of glutathione-related enzymes. *J. Nutr.* **2004**, *134*, 785–791.
98. Visioli, F.; Bellomo, G.; Montedoro, G.; Galli, C. Low density lipoprotein oxidation is inhibited *in vitro* by olive oil constituents. *Atherosclerosis* **1995**, *117*, 25–32.
99. Cooke, M.S.; Evans, M.D.; Dizdaroglu, M.; Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *Fed. Am. Soc. Exper. Biol. J.* **2003**, *17*, 1195–1214.
100. Machowetz, A.; Poulsen, H.E.; Gruendel, S.; Weimann, A.; Fito, M.; Marrugat, J.; de la Torre, R.; Salonen, J.T.; Nyyssonen, K.; Mursu, J.; Nascetti, S.; Gaddi, A.; Kiesewetter, H.; Baumler, H.; Selmi, H.; Kaikkonen, J.; Zunft, H.J.; Covas, M.I.; Koebnick, C. Effect of olive oils on

- biomarkers of oxidative DNA stress in Northern and Southern Europeans. *FASEB J.* **2007**, *21*, 45–52.
101. Jacomelli, M.; Pitozzi, V.; Zaid, M.; Larrosa, M.; Tonini, G.; Martini, A.; Urbani, S.; Taticchi, A.; Servili, M.; Dolara, P.; Giovannelli, L. Dietary extra-virgin olive oil rich in phenolic antioxidants and the aging process: long-term effects in the rat. *J. Nutr. Biochem.* **2009**, doi:10.1016/j.jnutbio.2008.12.014.
 102. Quiles, J.L.; Farquharson, A.J.; Simpson, D.K.; Grant, I.; Wahle, K.W. Olive oil phenolics: effects on DNA oxidation and redox enzyme mRNA in prostate cells. *Br. J. Nutr.* **2002**, *88*, 225–234.
 103. Fabiani, R.; Rosignoli, P.; De Bartolomeo, A.; Fuccelli, R.; Servili, M.; Montedoro, G.F.; Morozzi, G. Oxidative DNA damage is prevented by extracts of olive oil, hydroxytyrosol, and other olive phenolic compounds in human blood mononuclear cells and HL60 cells. *J. Nutr.* **2008**, *138*, 1411–1416.
 104. Reinisch, N.; Kiechl, S.; Mayr, C.; Schratzberger, P.; Dunzendorfer, S.; Kahler, C.M.; Buratti, T.; Willeit, J.; Wiedermann, C.J. Association of high plasma antioxidant capacity with new lesion formation in carotid atherosclerosis: a prospective study. *Eur. J. Clin. Invest.* **1998**, *28*, 787–792.
 105. Goya, L.; Mateos, R.; Bravo, L. Effect of the olive oil phenol hydroxytyrosol on human hepatoma HepG2 cells: Protection against oxidative stress induced by tert-butylhydroperoxide. *Eur. J. Nutr.* **2007**, *46*, 70–78.
 106. De la Puerta, R.; Martinez Dominguez, M.E.; Ruiz-Gutierrez, V.; Flavill, J.A.; Hoult, J.R. Effects of virgin olive oil phenolics on scavenging of reactive nitrogen species and upon nitrenergic neurotransmission. *Life Sci.* **2001**, *69*, 1213–1222.
 107. Owen, R.W.; Giacosa, A.; Hull, W.E.; Haubner, R.; Spiegelhalder, B.; Bartsch, H. The antioxidant/anticancer potential of phenolic compounds isolated from olive oil. *Eur. J. Cancer* **2000**, *36*, 1235–1247.
 108. Paiva-Martins, F.; Fernandes, J.; Rocha, S.; Nascimento, H.; Vitorino, R.; Amado, F.; Borges, F.; Belo, L.; Santos-Silva, A. Effects of olive oil polyphenols on erythrocyte oxidative damage. *Mol. Nutr. Food Res.* **2009**, *53*, 609–616.
 109. Moreno, J.J. Effect of olive oil minor components on oxidative stress and arachidonic acid mobilization and metabolism by macrophages. *Free Rad. Biol. Med.* **2003**, *35*, 1073–1081.
 110. Biswas, S.K.; Newby, D.E.; Rahman, I.; Megson, I.L. Depressed glutathione synthesis precedes oxidative stress and atherogenesis in Apo-E(-/-) mice. *Biochem. Biophys. Res. Comm.* **2005**, *338*, 1368–1373.
 111. Visioli, F.; Wolfram, R.; Richard, D.; Abdullah, M.I.C.B.; Crea, R. Olive phenolics increase glutathione levels in healthy volunteers. *J. Agric. Food Chem.* **2009**, *57*, 1793–1796.
 112. Loru, D.; Incani, A.; Deiaa, M.; Corona, G.; Atzeri, A.; Melis, M.P.; Rosa, A.; Dessi, M.A. Protective effect of hydroxytyrosol and tyrosol against oxidative stress in kidney cells. *Toxicol. Ind. Health* **2009**, *25*, 301–310.
 113. Packard, R.R.S.; Libby, P. Inflammation in atherosclerosis: From vascular biology to biomarker discovery and risk prediction. *Clin. Chem.* **2008**, *54*, 24–38.

114. Groff, J.L.; Gropper, S.S. *Advanced Nutrition and Human Metabolism*, 3rd ed.; Wadsworth Thomson Learning: Belmont, CA, USA, 2000.
115. Tiziani, A. *Harvard's Nursing Guide to Drugs*; Elsevier Australia: Sydney, Australia, 2006.
116. Leger, C.L.; Carbonneau, M.A.; Michel, F.; Mas, E.; Monnier, L.; Cristol, J.P.; Descomps, B.A. thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *Eur. J. Clin. Nutr.* **2005**, *59*, 727–730.
117. Beauchamp, G.K.; Keast, R.S.; Morel, D.; Lin, J.; Pika, J.; Han, Q.; Lee, C.H.; Smith, A.B.; Breslin, P.A. Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. *Nature* **2005**, *437*, 45–46.
118. Brody, T. *Nutritional Biochemistry*, 2nd ed.; Academic Press: San Diego, CA, USA, 1999.
119. White, J.G. Platelets and atherosclerosis. *Eur. J. Clin. Invest.* **1994**, *24* (Suppl 1), 25–29.
120. De La Cruz, J.P.; Villalobos, M.A.; Carmona, J.A.; Martin-Romero, M.; Smith-Agreda, J.M.; de la Cuesta, F.S. Antithrombotic potential of olive oil administration in rabbits with elevated cholesterol. *Thromb. Res.* **2000**, *100*, 305–315.
121. Perona, J.S.; Cabello-Moruno, R.; Ruiz-Gutierrez, V. The role of virgin olive oil components in the modulation of endothelial function. *J. Nutr. Biochem.* **2006**, *17*, 429–445.
122. Togna, G.I.; Togna, A.R.; Franconi, M.; Marra, C.; Guiso, M. Olive oil isochromans inhibit human platelet reactivity. *J. Nutr.* **2003**, *133*, 2532–2536.
123. Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedoro, G.; Galli, C. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* **1995**, *78*, 151–160.
124. Dell' Agli, M.; Maschi, O.; Galli, G. V.; Fagnani, R.; Dal Cero, E.; Caruso, D.; Bosisio, E. Inhibition of platelet aggregation by olive oil phenols via cAMP-phosphodiesterase. *Br. J. Nutr.* **2008**, *99*, 945–951.
125. Manna, C.; Napoli, D.; Cacciapuoti, G.; Porcelli, M.; Zappia, V. Olive oil phenolic compounds inhibit homocysteine-induced endothelial cell adhesion regardless of their different antioxidant activity. *J. Agric. Food Chem.* **2009**, *57*, 3478–3482.
126. Evan, G.I.; Vousden, K.H. Proliferation, cell cycle and apoptosis in cancer. *Nature* **2001**, *411*, 342–348.
127. Fabiani, R.; De Bartolomeo, A.; Rosignoli, P.; Servili, M.; Selvaggini, R.; Montedoro, G.F.; Di Saverio, C.; Morozzi, G. Virgin olive oil phenols inhibit proliferation of human promyelocytic leukemia cells (HL60) by inducing apoptosis and differentiation. *J. Nutr.* **2006**, *136*, 614–619.
128. Gill, C.I.; Boyd, A.; McDermott, E.; McCann, M.; Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G.; McGlynn, H.; Rowland, I. Potential anti-cancer effects of virgin olive oil phenols on colorectal carcinogenesis models *in vitro*. *Int. J. Cancer* **2005**, *117*, 1–7.
129. Fini, L.; Hotchkiss, E.; Fogliano, V.; Graziani, G.; Romano, M.; De Vol, E.B.; Qin, H.; Selgrad, M.; Boland, C.R.; Ricciardiello, L. Chemopreventive properties of pinoretinol-rich olive oil involve a selective activation of the ATM-p53 cascade in colon cancer cell lines. *Carcinogenesis* **2008**, *29*, 139–146.
130. Hashim, Y.Z.; Rowland, I.R.; McGlynn, H.; Servili, M.; Selvaggini, R.; Taticchi, A.; Esposto, S.; Montedoro, G.; Kaisalo, L.; Wahala, K.; Gill, C.I. Inhibitory effects of olive oil phenolics on invasion in human colon adenocarcinoma cells *in vitro*. *Int. J. Cancer* **2008**, *122*, 495–500.

131. Corona, G.; Deiana, M.; Incani, A.; Vauzour, D.; Dessi, M.A.; Spencer, J.P.E. Hydroxytyrosol inhibits the proliferation of human colon adenocarcinoma cells through inhibition of ERK1/2 and cyclin D1. *Mol. Nutr. Food Res.* **2009**, *53*, 897–903.
132. Menendez, J.A.; Vazquez-Martin, A.; Colomer, R.; Brunet, J.; Carrasco-Pancorbo, A.; Garcia-Villalba, R.; Fernandez-Gutierrez, A.; Segura-Carretero, A. Olive oil's bitter principle reverses acquired autoresistance to trastuzumab (Herceptin) in HER2-overexpressing breast cancer cells. *BMC Cancer* **2007**, *7*, 80–99.
133. Menendez, J.A.; Vazquez-Martin, A.; Oliveras-Ferraro, C.; Garcia-Villalba, R.; Carrasco-Pancorbo, A.; Fernandez-Gutierrez, A.; Segura-Carretero, A. Analysing effects of extra-virgin olive oil polyphenols on breast cancer-associated fatty acid synthase protein expression using reverse-phase protein microarrays. *Int. J. Mol. Med.* **2008**, *22*, 433–439.
134. Menendez, J.A.; Vazquez-Martin, A.; Oliveras-Ferraro, C.; Garcia-Villalba, R.; Carrasco-Pancorbo, A.; Fernandez-Gutierrez, A.; Segura-Carretero, A. Extra-virgin olive oil polyphenolics inhibit HER2 (erbB-2)-induced malignant transformation in human breast epithelial cells: Relationship between the chemical structures of extra-virgin olive oil secoiridoids and lignans and their inhibitory activities on the tyrosine kinase activity of HER2. *Int. J. Oncol.* **2009**, *34*, 43–51.
135. Han, J.; Talorete, T.P.N.; Yamada, P.; Isoda, H. Anti-proliferative and apoptotic effects of oleuropein and hydroxytyrosol on human breast cancer MCF-7 cells. *Cytotechnology* **2009**, *59*, 45–53.
136. Manna, C.; Galletti, P.; Cucciolla, V.; Moltedo, O.; Leone, A.; Zappia, V. The protective effect of the olive oil polyphenol (3,4-dihydroxyphenyl)-ethanol counteracts reactive oxygen metabolite-induced cytotoxicity in Caco-2 cells. *J. Nutr.* **1997**, *127*, 286–292.
137. Li, W.; Sperry, J.B.; Crowe, A.; Trojanowki, J.Q.; Smith, A.B.; Lee, V.M.Y. Inhibition of tau fibrillization by oleocanthal via reaction with amino groups of tau. *J. Neurochem.* **2009**, *110*, 1339–1351.
138. Pitt, J.; Roth, W.; Lacor, P.; Blankenship, M.; Velasco, P.; De Felice, F.; Breslin, P.A.; Klein, W.L. Alzheimer's-associated A-beta oligomers show altered structure, immunoreactivity and synaptotoxicity with low doses of oleocanthal. *Toxicol. Appl. Pharmacol.* **2009**, doi:10.1016/j.taap.2009.07.018.
139. Gonzalez-Correa, J.A.; Navas, M.D.; Lopez-Villodres, J.A.; Trujillo, M.; Espartero, J.L.; De la Cruz, J.P. Neuroprotective effect of hydroxytyrosol and hydroxytyrosol acetate in rat brain slices subjected to hypoxia-reoxygenation. *Neurosci. Lett.* **2008**, *446*, 143–146.
140. Medina, E.; de Castro, A.; Romero, C.; Brenes, M. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. *J. Agric. Food Chem.* **2006**, *54*, 4954–4961.
141. Romero, C.; Medina, E.; Vargas, J.; Brenes, M.; De Castro, A. *In vitro* activity of olive oil polyphenols against *Helicobacter pylori*. *J. Agric. Food Chem.* **2007**, *55*, 680–686.
142. Bisignano, G.; Tomaino, A.; Lo Cascio, R.; Crisafi, G.; Uccella, N.; Saija, A. On the in-vitro antimicrobial activity of oleuropein and hydroxytyrosol. *J. Pharma. Pharmacol.* **1999**, *51*, 971–974.

143. Puel, C.; Mardon, J.; Agalias, A.; Davicco, M.J.; Lebecque, P.; Mazur, A.; Horcajada, M.N.; Skaltsounis, A.L.; Coxam, V. Major phenolic compounds in olive oil modulate bone loss in an ovariectomy/inflammation experimental model. *J. Agric. Food Chem.* **2008**, *56*, 9417–9422.

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