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Health effects of olive oil polyphenols: Recent advances and possibilities for the use of health claims

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LIST OF ABBREVIATIONS

ALP	alkaline phosphatase
EFSA	European Feed Safety Authority
FDA	Food and Drug Administration
FVIIa	activated coagulation factor VII
GIT	gastro-intestinal tract
HDL	high density lipoprotein
LDL	low density lipoprotein
MD	Mediterranean diet
MSCs	mesenchymal stem cells
OOPC	olive oil phenolic compounds
PAI-1	plasminogen activator inhibitor type 1
PAMPs	pathogen-associated molecular patterns
PBMNCs	peripheral blood mononuclear cells
POLK	polymerase (DNA directed) kappa
PPARγ	peroxisome proliferator-activated receptor gamma
RCT	reverse cholesterol transport
ROS	reactive oxygen species
SCFA	short chain fatty acids
TF	tissue factor
t-PA	tissue plasminogen activator
VOO	virgin olive oil

KEYWORDS

olive oil, polyphenols, health, health claims, labelling

ABSTRACT

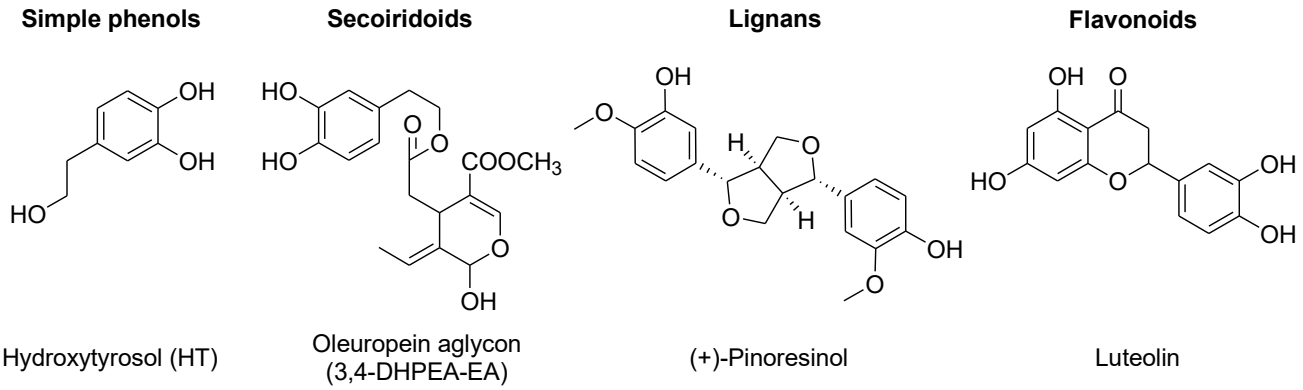
The Mediterranean diet and consumption of olive oil have been connected in several studies with longevity and a reduced risk of morbidity and mortality. Life-style, such as regular physical activity, a healthy diet, and the existing social cohesion in Southern European countries have been recognised as candidate protective factors which may explain the Mediterranean Paradox. Along with some other characteristics of the Mediterranean diet, the use of olive oil as the main source of fat is common in Southern European countries. The benefits of consuming olive oil have been known since antiquity and were traditionally attributed to its high content in oleic acid. However, is now well established that these effects must also be attributed to the phenolic fraction of olive oil with its anti-oxidant, anti-inflammatory and anti-microbial activities. The mechanisms of these activities are varied and probably inter-connected. For some activities of olive oil phenolic compounds (OOPC) the evidence is already strong enough to enable the legal use of health claims on foods. This review discusses the health effects of olive oil phenols along with the possibilities of communicating these effects on food labels.

1. INTRODUCTION

In general, countries from Southern Europe present the lowest values of accumulated incidence of myocardial infarction [1]. The candidate protective factors which may explain this Mediterranean Paradox can be life-style factors such as regular physical activity, a healthy diet, and the existing social cohesion in Southern European countries. Since Ancel Keys presented the Mediterranean Diet as a health protecting one [2], there have been many studies indicating a link between adherence to the Mediterranean diet and a reduced risk of overall mortality, cardiovascular mortality, cancer incidence and mortality, as well as the incidence of neurodegenerative diseases [3-7]. The Mediterranean diet (MD) is characterised by: a) the high consumption of vegetables, legumes, fruit, and cereals; b) regular, but moderate, wine intake; c) the moderate consumption of fish and white meat; d) a moderate intake of dairy products; e) the low consumption of red meat; and f) a relatively high fat consumption (up to 40% of total energy intake), mostly from monounsaturated fatty acids (up to 20% of energy) mainly provided by olive oil, the principal source of culinary (especially dressing) fat [8]. However, olive oil consumption in Mediterranean countries varies within countries. As an example, the three major producers of olive oil (Spain, Greece, and Italy) consumed different amounts of olive oil per capita in 2011: 13.4 L, 10.7 L and 21.3 L respectively [9]. The benefits of consuming olive oil have been known since antiquity and extensively reported [10]. Traditionally, the health effects of olive oil were attributed to its high content in oleic acid. Nowadays, scientific knowledge has demonstrated that these effects must also be attributed to the phenolic fraction of olive oil [11, 12]. This fraction has been shown to support anti-oxidant, anti-inflammatory and anti-microbial activities. The mechanisms by which OOPC can support these activities are varied and, most probably, inter-connected. On one hand, due to their anti-oxidant capacity, such as cell redox state modulating enzyme systems, OOPC act as the first line of defence against free radicals in cellular compartments, and also extracellularly [13]. Current evidence indicates oxidative damage is a promoter of pathophysiological changes occurring in oxidative stress-associated diseases like coronary heart disease, cancer, and neurodegenerative pathologies, along with ageing [14-16]. Further, OOPC are able to modulate gene expression, influencing gene and protein expression and, subsequently, metabolite production.

The phenolic composition of olive oils varies in quantity (50-800 mg/L) and quality depending on the olive variety, the age of the tree, agricultural techniques used in cultivation,

degree of ripeness, soil composition, climate, the processing technique and storage [17-21]. The chemical composition of the phenolic fraction of olive oil has been studied extensively [22-27]. Phenolic compounds of olive oil are conventionally called polyphenols, although not all of them are poly-hydroxyl derivatives. In general, four major classes of olive oil phenolic compounds (OOPC) can be found in these oils: flavonoids, lignans, simple phenols, and secoiridoids (Scheme 1). The levels of the last two groups are the most important and many of such OOPC can be found exclusively in olive oils [19]. The absorption and metabolism of various OOPC vary; they are regarded as highly bioavailable due to their good water-solubility, with an absorption efficiency of about 55–66 mol% [28]. In vitro experiments show that OOPC are efficiently absorbed from the intestine through a pathway which is not dependent on chylomicron formation.



Scheme 1: *Main classes of OOPC with representative compounds*

While reviews are available on the biological properties of OOPC [20, 29-37], we mostly focused on recent advances along with the possibility of using substantiated health claims in the marketing of olive oil.

Besides the reported benefits, a change in the Mediterranean diet pattern towards a more Western one has been observed. Possible reasons are globalisation, changes in life-style and price increases of some of the major components of the Mediterranean diet such as olive oil and vegetables. These changes, in turn, could influence negatively in the ‘healthy status’ of Mediterranean countries [38].

2. SELECTION OF STUDIES AND RATIONALE

A literature review was carried out in MEDLINE up until September 2012. The search was performed for experimental and human studies assessing the effect, either acute or sustained, of different concentrations of OOPC on human health. The following Medical Subject Heading Terms were used: Olive oil polyphenols, hydroxytyrosol, oleuropein, olive oil, phenol extract, oxidative stress, inflammation, platelet aggregation, endothelial dysfunction, lipoproteins, cholesterol efflux, LDL oxidation, DNA oxidation, gene expression, atherosclerosis, coronary heart disease, cancer, ageing, gut microbiota. The selection of the studies presented in this review is based on their quality and focus on the beneficial health effects of OOPC. The selection of the studies for this review was based on the idea of providing evidence for health claims, as well as on the quality of the studies. From the 81 articles initially retrieved, 66 were given a particular focus: 28 studies with cell cultures, 13 animal studies, and 25 studies on humans. When evidence from experimental studies is referred to, those studies performed in cell cultures included a control and the results represented the mean of at least three experiments. For animal models, experiments were performed with at least four animals per group, with the presence of a control group and a parallel or cross-over design. Selected human studies were randomised and controlled intervention trials with parallel or cross-over designs. The significance of the results presented was with $P<0.05$. From the 15 articles revised but not included in this review, seven human studies were not randomised ones. In four human studies the changes in health markers did not achieve statistical significance versus the control group [39-42]. In one animal study, the high hydroxytyrosol doses administered promoted atherosclerosis in Apo-E deficient mice [43]. In three *in vitro* studies with cell cultures, experiments were not carried out in triplicate.

3. PROTECTION AGAINST OXIDATIVE DAMAGE AND INFLAMMATION

One of the main properties of OOPC contributing directly or indirectly to their beneficial health effects is their anti-oxidant capacity. A series of reviews covering this topic is available [20, 29, 33, 35, 36, 44]. Amongst others, the targets for reactive oxygen species (ROS) are lipids, DNA and proteins [45-48]. Most ROS are naturally produced by our organism, such as those derived from oxygen in the aerobic metabolism. Others enter our body through food or the environment. Oxidation and inflammation are intertwined processes. Besides promoting oxidative damage, free radicals activate pro- and anti-inflammatory cytokines. Extra VOO components have been reported as having a strong anti-inflammatory effect both *in vitro* and

in vivo [32, 49] described by some authors as comparable to that of ibuprofen [50]. Recently, the in vivo inhibition of NFkB, an important link between oxidation and inflammation in the postprandial state by oils rich in phenols (0.45 mL/kg body weight of olive oil containing 400 ppm of OOPC) has been demonstrated in obese individuals [51]. Both oxidation and inflammation are involved in the development of a wide variety of chronic degenerative pathologies such as cardio-vascular diseases, cancer or ageing-related diseases, amongst others [52-54].

Cardiovascular risk factors

OOPC have been shown to be beneficial in the prevention and/or treatment of risk factors for cardiovascular diseases. Most of these diseases have in common the development of atherosclerotic plaque in the endothelium of the blood vessels. Put briefly, this plaque is formed by the accumulation of cholesterol inside the macrophages that reside in the intima which generates an inflammatory response that eventually triggers acute thrombotic vascular disease, including myocardial infarction, stroke, and sudden cardiac death [55]. When oxidised, low density lipoprotein (LDL) and high density lipoprotein (HDL) influence the cholesterol charge of macrophages [56]. Oxidised LDL is able to load cholesterol into the macrophages [57]. Several human trials have demonstrated that the degree of oxidised LDL decreases as the phenolic content in the administered olive oil increases, from 0 to 800 mg/kg [58-63]. More recently, Vázquez-Velasco et al. observed a decrease in the level of oxidised LDL when hydroxytyrosol-enriched sunflower oil taken daily for 3 weeks (45-50 mg/d) was administered to healthy volunteers [64]. In 40 women suffering from stable coronary heart disease (CHD), olive oil enriched with OOPC (161 mg/kg) was able to decrease the levels of oxidised LDL in plasma compared with another olive oil with a smaller amount of OOPC (15 mg/kg) [65].

On the basis of well-defined clinical intervention studies [61-63] a cause-and-effect relationship has been established between the consumption of OOPC and the protection of LDL particles from oxidative damage. This was the subject of a recent scientific evaluation of a health claim application by the European Food Safety Authority (EFSA) [66]. It was recently observed in a clinical intervention study that the effect of OOPC (25 mL/day of 3 olive oils with high (366 mg/kg), medium (164 mg/kg), and low (2.7 mg/kg) phenolic content) on oxidised LDL could be due to oxidised LDL antibodies [67]. In the EUROLIVE study, 200 healthy, non-smoking males were recruited in six centres of five European countries. The volunteers were given 25 mL per day of raw olive oil with high (366 mg/kg),

medium (164 mg/kg), and low (3 mg/kg) phenolic content in a randomised, cross-over, double-blind and controlled trial, with a Latin square for three treatments in the cross-over randomised trial. There was a wash-out period of 2 weeks before each olive oil intervention, with the intervention periods lasting 3 weeks. Not only was a decrease in *in vivo* lipid oxidative damage observed, but an increase in HDL cholesterol was also noted in a direct, dose-dependent manner with the phenolic content of the olive oil. This increase in HDL levels with OOPC has been observed in other human studies [61-63]. An increase in HDL cholesterol is one of the goals of current cardiovascular disease therapies [68]. However, the beneficial effects of OOPC in relation to the maintenance of normal blood HDL-cholesterol concentrations have been recently evaluated by the EFSA, which concluded that the provided evidence was insufficient to establish a cause-and-effect relationship [69]. Recent studies show that the functionality of HDL must be of greater importance than its amount [68, 70-72]. This functionality of the HDL particle could be related to the promotion of cholesterol efflux from macrophages in the so-called "reverse cholesterol transport" (RCT) [73-76]. It has been observed that the oxidation of HDL influences its functionality [77-79] and OOPC could be able to counteract this oxidation. The administration of olive oil with 400 ppm of OOPC has also been demonstrated to improve endothelial function in hypercholesterolemic patients [80] in the postprandial state and in patients with early atherosclerosis after 4 months with a daily dose of olive oil containing 340 mg/kg of OOPC [81]. Moreno-Luna and co-workers also observed an improvement in endothelial function in 24 young women with high-normal blood pressure or stage 1 essential hypertension [82]. Women were given 30 mg/day of OOPC-rich olive oil and OOPC-free olive oil in a double-blind, randomised, crossover dietary-intervention study. In addition, they found a decrease in blood pressure. Recent *in vitro* studies point to the beneficial effects of hydroxytyrosol [83-85] and its glucuronidated metabolites [86] as being able to inhibit the oxidative damage caused by various reactive oxygen species such as H_2O_2, which is a major cause of endothelial dysfunction [87]. OOPC have been shown to influence platelet aggregation, which is a key factor in the development of thrombus and myocardial infarction or angina. DHPE (2-(3,4-di-hydroxyphenyl)-ethanol), an olive oil phenol, has shown to interfere with platelet aggregation *in vitro* in a concentration of 400 mM [88]. This activity has also been compared with that of acetyl salicylic acid [89]. In two randomised crossover studies, virgin olive oil, rich in polyphenols (50 mL olive oil, 607 ppm OOPC), was shown to be more effective in lowering LTB4 and TXB2 than refined olive oil, with a low phenolic content (50 mL, 16 ppm OOPC) at postprandial state in healthy subjects [90]. This effect was also observed after sustained consumption in mildly

dyslipidemic patients (6.64 vs 0.08 mg OOPC per day)[91]. Concerning haemostatic factors, a protective pattern emerges from two clinical intervention studies in which the effect of olive oil phenolic compounds was tested. The acute intake of phenol-rich virgin olive oil (40 mL of 400 ppm OOPC) has been associated with a smaller postprandial increase of tissue factor (TF), activated coagulation factor VII (FVIIa), and fibrinogen, as well as a greater decrease of the fibrinolysis pathway factors such as tissue plasminogen activator (t-PA) and plasminogen activator inhibitor type 1 (PAI-1) in both healthy and hypercholesterolemic subjects compared with the intake of a low-phenol virgin olive oil (40 mL of 80 ppm OOPC) [92]. In smooth muscle cells, hydroxytyrosol has shown to promote apoptosis [83] and oleuropein [93] to inhibit their proliferation.

Cancer risk

Epidemiological studies show an inverse association between olive oil intake and the occurrence of different types of cancer [94-97]. The contribution of OOPC is thought to be mediated by their influence on the redox and inflammation status, and the cell functions. *In vivo* [61, 98] and *in vitro* [99] studies reveal the protective effect of OOPC on DNA oxidatio. Ilavarsi recently reported that hydroxytyrosol (100μM) was able to increase cell viability, normalising the antioxidant enzymes and decreasing levels of ROS in human peripheral blood mononuclear cells treated with 2,3,7,8-Tetrachlorodibenzo-p-dioxin [100]. OOPC are able to interfere with basic cell functions that interfere with the cell cycle. Hydroxytyrosol interfered with the G1 cell cycle and apoptosis in human colon adenocarcinoma cells and promyelocytic leukaemia cells at concentrations ranging from 50 to 100μM [101]. Also in human adenocarcinoma cells, hydroxytyrosol (50 mg/mL) inhibited the p38/CREB phosphorylation and COX-2 expression [102] and arrested at G2/M and inhibited cell growth in a dose-dependent manner (5-200μM) [103]. Oleuropein and hydroxytyrosol have also been shown to reduce the inflammatory angiogenesis in human vascular endothelial cells in concentrations ranging from 0.1-50 μM [104]. Mechanisms proposed for the observed effects are: the interaction with steroid and growth factor receptor-mediated functions, interaction with specific protein kinases and oncogenes/oncoproteins, inhibition of enzymes related to tumour promotion and metastasis or a direct effect on nucleic acids and nucleoproteins [105]. These experimental studies indicate the potential beneficial effect of OOPC within the context of a healthy diet as a useful tool in the prevention of cancer. Nevertheless, further studies in humans are warranted.

Age-related processes

According to the free-radical theory [106], the ageing process is the result of accumulated oxidative injury throughout one's lifetime. Recently, Farr et al. found that extra VOO (210 mg OOPC/kg) has beneficial effects on learning and memory deficits found in ageing and diseases, such as those related to the overproduction of Amyloid-β peptide [107]. In this study, OOPC were able to reverse oxidative damage in the brain of SAMP8 mice, an age-related learning/memory impairment model associated with an increased amyloid-β protein and brain oxidative damage. This effect was augmented by increasing concentrations of OOPC in extra VOO (from 210 to 1050 mg/kg). Moreover, St-Laurent-Thibault et al. found that tyrosol and hydroxytyrosol (100 μg/mL) could serve as neuroprotective agents against Aβ-peptide in cultured neuroblastoma N2a cells [108]. Tau proteins are mainly expressed in the neurons of the central nervous system, playing a key structural role in the distal portion of axons [109]. With Alzheimer's disease and related tauopathies, Tau aggregation in fibrillary tangles contributes to intraneuronal and glial lesions [110]. It was recently reported that hydroxytyrosol, oleuropein and especially oleuropein aglycon are able to act as Tau aggregation inhibitors at low (10 μM) concentrations *in vitro* [111].

Age-related macular degeneration, which is characterised by a degeneration of the retinal pigment epithelium and the photoreceptors in the macular area of the retina, eventually causes blindness in most cases. Studies on ARPE-19 human retinal pigment epithelial cells have demonstrated that hydroxytyrosol (100 μmol/L) is able to prevent the degeneration of these cells by the induction of phase II detoxifying enzymes and the enhancement of mitochondrial biogenesis [112].

Other chronic inflammatory disorders

In a model of mice subjected to collagen-induced rheumatoid arthritis, oleuropein aglycon (40 μg/kg) exerted an anti-inflammatory effect and ameliorated the associated tissue damage [113]. It also caused a significant reduction of all the parameters of inflammation measured in a model of carrageenan-induced pleurisy [114] when administered 30 min after the challenge with carrageenan. These studies indicate the potential benefits of incorporating OOPC in the diet.

4. EFFECTS ON HUMAN MICROBIOTA

The ingestion of phenolic compounds from olive oil can also influence the gut microbial balance since most of them are not completely absorbed into the upper parts of the gastro-intestinal tract (GIT) and reach the lower parts of the GIT where they are metabolised by the gut microflora [115]. Since it is known that pathogens in the gut can modulate inflammatory signalling pathways [116], the anti-microbial activity of the phenolic compounds in the gut could serve as a tool to counter atherosclerosis development. Phenolic compounds can also selectively stimulate the growth of beneficial bacteria, such as *Lactobacillus* [117]. Apart from the benefits of these types of bacteria [118], recent research offers strong evidence suggesting that these bacteria influence lipid metabolism, lowering cholesterol levels [119]. The ways in which these bacteria influence the cholesterol metabolism are not fully understood and comprise more than one mechanism. Studies by Christianes et al. show that intestinal lactobacilli possess genes encoding bile-salt hydrolase [120]. This enzyme deconjugates bile acids, preventing them from being reabsorbed, resulting in the excretion of larger amounts of free bile acids in faeces [121, 122]. This would be a major pathway for the elimination of cholesterol. As the synthesis of new bile acids rises in compensation, the uptake of LDL by hepatic apo B:E receptors is up-regulated and blood cholesterol levels fall [123].

It has also been suggested that lactobacilli can assimilate cholesterol from media during growth [124] or even non-growing and dead lactobacilli [125] by binding onto the bacterial cellular surface, leading to reduced serum cholesterol levels. Further, the reported capacity of some bacteria such as *L. acidophilus* to bind bile salts [126] can also inhibit the formation of intestinal cholesterol micelle; disrupted micelles cannot transport fatty acids to the intestinal mucosal surface for absorption, leading to a reduced cholesterol level [127]. Last but not least, it is possible that increased populations of *Bifidobacterium* spp., *Lactobacillus* spp. and

Enterococcus spp., microorganisms that exhibit immunoregulatory properties, may be linked to the development of the atherosclerotic lesion [128] since atherosclerosis is a chronic immune inflammatory disease. For example, Kalsson observed an increase in bacterial diversity after including *L. plantarum* DSM 9843 in the diet, which was related with a decrease in the translocation of PAMPs (pathogen-associated molecular patterns) that negatively affects atherosclerosis [129].

The use of polyphenols by gut bacteria generates bioactive phenolic metabolites. Hydroxytyrosol is partly metabolised to homovanillyl alcohol [115, 130] which is itself able to have an antioxidant action. The metabolism of gut bacteria also produces short chain fatty acids (SCFA) which can influence the cholesterol metabolism. Several studies show that acetate increases systemic total cholesterol while propionate reduces the hypercholesterolemic effect caused by acetate [131, 132]. Propionate is cleared by the liver and has been suggested to affect the cholesterol metabolism [133]. Butyrate is known to inhibit liver cholesterol synthesis and provide a source of energy for human colon epithelial cells. Propionate is involved in inhibition of the synthesis of fatty acids in the liver, thereby lowering the rates of triacylglycerol secretion and the rate of cholesterol synthesis, which could lead to an improvement of circulating cholesterol levels [134]. Thus, phenolic compounds that reach the gut could modulate the microbial ecosystem in a beneficial sense for the host. In addition, OOPC have demonstrated their efficacy *in vitro* as inhibitors of a wide range of microbial gastrointestinal pathogens such as *Escherichia coli* [135] *or Helicobacter pylori* [136], respiratory pathogens such as *Haemophilus influenzae* [137] or *Mycoplasma pneumoniae* [138] dental pathogens like *Streptococcus mutans* [139] and genital pathogens such as *Candida albicans* [135] or even virus like herpes mononucleosis [140] and the para-influenza type 3 virus [141]. The inhibitory effect of OOPC on bacteria found in atherosclerotic plaque, such as *Chryseomonas, Veillonella and Streptococcus,* which are thought to contribute to the atherosclerotic process [142] remains to be elucidated.

5. NUTRIGENOMIC EFFECTS

Dietary components can regulate the expression of genes in the transcription, mRNA processing, mRNA stability, and trans and post-translational modification stages. In this sense, besides their antioxidant and anti-inflammatory capacities OOPC are able to modify gene expression coding in a protective mode for proteins participating in the cellular mechanisms involved in oxidative stress resistance, inflammation or lipid metabolism amongst others.

Changes in gene expression may support the beneficial effects observed in phenotypic biomarkers for atherosclerotic processes following a Mediterranean diet and olive oil consumption. Within this context, a randomised trial was performed [143] with the aim of assessing the *in vivo* gene expression changes in the peripheral blood mononuclear cells of 90 healthy volunteers promoted by adherence to a Mediterranean diet (MD) with high or low-OOPC olive oil. The design of the study was a randomised, parallel, controlled, double-blind trial with three dietary interventions over three months. One group followed their habitual diet, and the other two groups followed an MD which in one group was supplemented with VOO (328 mg/kg OOPC) and in the other with washed olive oil (55 mg/kg OOPC). In both MD groups, 1L of olive oil per week was supplied for all the family. When changes in gene expression between the MD pattern and the control group were analysed, several genes related to inflammation (Rho-GTPase-activating protein 15, interferon gamma, and interleukin 7 receptor) or related with oxidative stress (adrenergic beta 2 receptor, and polimerasa kappa) showed a significant expression change. A significant linear decreasing trend was observed related to the phenolic content of the olive oil, in the expression of interferon gamma, the Rho-GTPase-activating protein 15, and the adrenergic beta 2 receptor. Thus, all of the effects, with the exception of *POLK* expression, were particularly observed when VOO was present in the MD pattern. This study demonstrates a down-regulation in the expression of atherosclerosis-related genes occurs in human PBMNCs after 3 months of an MD and points out the significant role of olive oil phenolic compounds in the down-regulation of atherosclerosis-related genes within the framework of an MD. Nutrigenomic science is hindered by the fact that gene expression changes are modest after an intervention with a diet pattern or a single food (in particular with real-life doses). Human intervention studies demonstrate that OOPC play a role in the down-regulation of pro-atherogenic genes in microarray analyses [143, 144].

Besides their anti-cancer activity due to their direct effect on DNA oxidative damage, OOPC are also able to act at the gene level. Recent *in vitro* studies show the capacity of OOPC to interact with signalling pathways controlling growth and differentiation in breast cancer studies, in both cell lines (2000 μg/mL) [145] and animal models (0.5 mg/kg body weight) [146].

In addition, OOPC have been shown to influence the expression of genes related to obesity. Warnke observed that hydroxytyrosol (25 μM) was able to modify genes related with adipocyte maduration and differentiation [147]. Drira reported that both hydroxytyrosol (100 and 150 μM) and oleuropein (200 and 300 μM) inhibited adipocyte differentiation by inhibiting the mitotic clonal expansion and by down-regulating adipogenesis-related genes

[148]. In animal models some unsaturated fatty alcohol derivatives of hydroxytyrosol, particularly hydroxytyrosol linoleylether (5 mg/kg), showed a hypophagic effect comparable to that of oleoylethanolamine, despite a lower affinity for the cannabinoid CB1 receptor and the PPARα receptor [149].

A diet intervention involving 737 participants has shown that an MD rich in VOO (1L/week) may reverse the effects of the-174G/C IL6 gene variant on 3-year body weight change [150]. In mesenchymal stem cells (MSCs), oleuropein (10^{-4} – 10^{-6}) induced a decrease in the expression of the genes involved in adipogenesis (the PPARγ, lipoprotein lipase, or fatty acid-binding protein 4), and minor fat accumulation [151]. Since the obesity process involves an increase in the number and/or size of adipocytes, these findings could pave the way for the potential use of OOPC in the management of obesity.

It is known that stimuli-inhibiting adipogenesis induces osteoblast differentiation, and vice versa [152]. Oleuropein has been shown to inhibit adipogenesis in MSCs from human bone marrow and, at the same time, to enhance their differentiation into osteoblasts [151]. The gene expression of osteoblastogenesis markers, RUNXII, osterix, collagen type I, osteocalcin, or alkaline phosphatase (ALP), was higher in osteoblast-induced, oleuropein-treated cells. The possible beneficial effect of this gene activation in mesenchymal stem cells on bone remodelling could be counteracted by the fact that these genes are also involved in vascular calcification during atherosclerotic plaque development. An in vivo cell specific effect of OOPC could account for the global protection provided by OOPC. Oleuropein and hydroxytyrosol inhibited the formation of multinucleated osteoclasts in culture, and oleuropein enhanced the deposition of calcium by osteoblasts. Further, oleuropein (50-100 μM) and hydroxytyrosol (10-100 μM) decreased bone loss from femurs in ovariectomised mice [153]. These findings could be useful in investigations for osteoporosis treatment research.

Sánchez-Fidalgo et al. observed an improvement of chronic colitis in female C57BL/6 mice with hydroxytyrosol (5 mg/kg body weight per day) through the down-regulation of the inducible enzyme nitric oxide synthase, plus its antioxidant/anti-inflammatory capacity [154].

6. HEALTH CLAIMS ON FOODS

The commercial communication of the beneficial effects of foods or their constituents on health by using health claims is a very convenient tool when it comes to marketing ‘healthy foods’ since consumers are very sensitive to health-related communications [155]. While

various countries take different approaches to ensure that health claims do not mislead consumers, the general approach is proper substantiation by generally accepted scientific data. The use of health claims in the European Union (EU) was harmonised in 2006 following the acceptance of a challenging and controversial regulation [156-161]. *A health claim* is defined as any claim that states, suggests or implies that a relationship exists between a food category, a food or one of its constituents and health. Scientific assessment is performed by the European Food Safety Authority (EFSA). Well-performed human intervention trials are particularly important for the successful substantiation of health claims and double-blind, randomised, placebo-controlled trials are considered the gold standard [161].

Several health claim applications for olive oil and its constituents have been assessed in recent years. Phenolic compounds naturally occurring in olive oil were shown [66] to significantly decrease the amount of circulating oxidised LDL particles *in vivo* in a dose-dependent manner in one large [63] and three smaller human intervention studies [61, 62, 130]; additional clinical and supportive evidence has also been provided [66]. On the basis of an established cause-and-effect relationship, the claim that "olive oil polyphenols contribute to the protection of blood lipids from oxidative stress" is now allowed on olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. The concentrations of OOPC in some olive oils may be too low to allow the consumption of such an amount of OOPC in the context of a balanced diet. The health claim concerning the role of OOPC in the maintenance of normal blood HDL-cholesterol has also been evaluated recently but the evidence provided was insufficient to establish a cause-and-effect relationship [69]. The use of a related health claim will not be possible without further data from well-controlled intervention studies. Some other claims for OOPC have also been evaluated in the last few years (i.e. the role in decreasing potentially pathogenic intestinal microorganisms, and anti-inflammatory properties), although stronger clinical evidence is needed before such claims may be used in commercial communications. However, some other claims not related to OOPC are currently possible for olive oils (**Table 1**).

Table 1: *General function health claims for olive oil constituents in which a cause-and-effect relationship has been confirmed by the European Food Safety Authority*

Olive oil constituent	**Health claim wording**	**Conditions of use**	**Ref.**

Olive oil polyphenols	Olive oil polyphenols contribute to the protection of blood lipids from oxidative stress.[1]	The claim may only be used for olive oil which contains at least 5 mg of hydroxytyrosol and its derivatives (e.g. oleuropein complex and tyrosol) per 20 g of olive oil. In order to bear the claim, information shall be given to the consumer that the beneficial effect is obtained with a daily intake of 20 g of olive oil.	[66]
Vitamin E	Vitamin E contributes to the protection of cells from oxidative stress.	The claim may only be used for food which is at least a source of vitamin E as referred to in the claim "source of vitamin". [2]	[162]
Monounsaturated fatty acids	Replacing saturated fats with unsaturated fats in the diet contributes to the maintenance of normal blood cholesterol levels.	The claim may only be used for foods where at least 45% of the fatty acids present in the product derive from unsaturated fat on the condition that unsaturated fat provides more than 20% of the energy of the product.	[163]

Note: [1]Health relationship: protection of LDL particles from oxidative damage; [2]Conditions for foods being a source of vitamin E in the EU: at least 18 mg vitamin E per L/kg.

7. LIMITATIONS

The beneficial effect of consuming olive oil can only be achieved in the context of a healthy dietary pattern and life-style, including physical activity. In practice, people consume meals and not individual foods or nutrients which has led to the holistic dietary approach to disease prevention. The high energy content of all oils represents the main limitation on recommendations for the increased consumption of any particular oil. The recommendations should not be to consume olive oil in addition to the usual diet, but to substitute other lipids with extra VOO, always aiming to strike a balance between energy intake and expenditure, preferably at a higher rather than a lower level [164]. In order to export guidelines on olive oil consumption, more and better designed studies are required in order to use them in a meta-analysis.

8. CONCLUSIONS

Despite the huge number of studies performed on OOPC and health, it is only regarding a few issues that there is enough knowledge to provide a whole body of scientific evidence. Health claims to be used on foods should be based on evidence-based medicine, together with data concerning the plausible mechanisms by which a food or food constituents could benefit human health. Concerning OOPC, there is a broad spectrum of experimental studies, *in vitro* or in animal models, on different issues related to health. However, these experimental studies are useful for generating hypotheses or testing possible mechanisms, but they do not provide scientific evidence of health benefits at the highest possible level. Each experimental issue for which promising results are obtained, must be followed by proper human randomised controlled studies. This link has only been fully accomplished in the case of LDL oxidation, inflammation, and partially on an increase of HDL cholesterol and an anti-thrombotic effect. Of all these issues, at present only the beneficial effect of OOPC-rich olive oil (providing a daily amount of at least 5 mg of hydroxytyrosol and its derivatives) on preventing the oxidation of LDL has been accepted as a health claim in the European Union.

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