Vascular Pharmacology 63 (2014) 127–134

Contents lists available at ScienceDirect

Vascular Pharmacology

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

Review

Vascular effects of the Mediterranean diet—Part II: Role of omega-3 fatty acids and olive oil polyphenols

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ARTICLE INFO

Article history:
Received 22 April 2014
Received in revised form 20 June 2014
Accepted 3 July 2014
Available online 16 October 2014

Keywords:
Mediterranean diets
ω-3 polyunsaturated fatty acids
Olive oil polyphenols
Inflammation
Nutrigenomics

ABSTRACT

The lower occurrence of cardiovascular disease and cancer in populations around the Mediterranean basin as detected in the 1950s was correctly attributed to the peculiar dietary habits of those populations. Essentially, until the mid-20th century, typical Mediterranean diets were rich in fruits, vegetables, legumes, whole-wheat bread, nuts, fish, and, as a common culinary trait, the routine use of extra-virgin olive oil. Nowadays, the regular adoption of such dietary patterns is still thought to result in healthful benefits. Such patterns ensure the assumption of molecules with antioxidant and anti-inflammatory actions, among which ω-3 polyunsaturated fatty acids (PUFAs), ω-9 monounsaturated fatty acids (oleic acid), and phenolic compounds. The aim of this review is to provide an update of the vasculo-protective pathways mediated by ω-3 PUFAs and polyphenols in the context of the modern Mediterranean dietary habits, including the possible cross-talk and synergy between these typical components. This review complements a parallel one focusing on the role of dietary nitrates and alimentary fats.

Contents

1. Premise and Introduction

The beneficial effects of Mediterranean diets have been reported as due to the synergistic interaction of various constituents. Numerous studies have demonstrated that single components, i.e., inorganic nitrates, monounsaturated fatty acids, ω-3 polyunsaturated fatty acids (PUFAs) and polyphenols, have specific roles in the prevention of inflammatory and degenerative diseases, as well as of cancer. The metabolic roles of inorganic nitrates and monounsaturated fatty acids have been previously reviewed (*Capurso C et al, Vascular Pharmacology in press*). In this review, the biochemical and mechanistic properties of ω-3 PUFAs and olive oil polyphenols in the context of the Mediterranean dietary habits are discussed as a basis for a possible interplay between these nutritional components.

After the striking protective effects reported for a Mediterranean-type diet in the secondary prevention of coronary heart disease [1], the recently documented benefits of a Mediterranean diet supplemented

DOI of original article: http://dx.doi.org/10.1016/j.vph.2014.10.001.
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http://dx.doi.org/10.1016/j.vph.2014.07.001
1537-1891/

in mixed nuts (as a source of ω-3 PUFAs besides to high-molecular-weight polyphenols, including proanthocyanidins and ellagitannins [2]) or extra-virgin olive oils (as a prominent source of low-molecular-weight polyphenols hydroxytyrosol and its derivatives, besides to monounsaturated fatty acids) in the primary prevention of major coronary events [3] also highlight the vasculo-protective potential of polyphenols and ω-3 PUFAs in the context of a Mediterranean dietary pattern. Such evidence, however, raises some questions, which will be addressed here: first, can the spontaneous adherence to a typical Mediterranean diet provide appreciable and biologically meaningful increases in plasma levels of polyphenols and ω-3 PUFAs? Second, are plant- and marine-derived polyphenols and ω-3 PUFAs endowed with shared or complementary vasculo-protective activities?

2. Role of ω-3 PUFAs in the Mediterranean diets

2.1. Structure, source and metabolism of ω-3PUFAs

In the last 50 years many epidemiological evidences have accumulated regarding the role of ω-3 PUFAs, namely α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the prevention and management of cardiovascular disease [4]. PUFAs are organic acids present in the diet and containing more than one double bonds in their aliphatic chain. Biologically relevant families of PUFAs are the omega-3 (ω-3) and the omega-6 (ω-6) PUFAs. Since the double bond in the ω-3 or ω-6 position cannot be inserted into FA by animal enzymes, but only by vegetable Δ12- and Δ15-desaturases, LA and ALA represent "essential fatty acids (EFAs)" for mammals [5]. Mammalian cells may indeed metabolize them (Fig. 1) through the action of a series of elongation and desaturation enzymes that insert double bonds into the molecules, with an additional final step for docosatetraenoic acid and DHA production that requires the translocation to peroxisomes for a β-oxidation reaction [6]. It is estimated that minimum human requirements are 1 and 0.2% of daily energy intake for ω-6 and ω-3 PUFAs, respectively [7]. Because LA and ALA are synthesized by plants, vegetables and vegetable oils are good sources of both FAs. Green plant tissues are especially rich in ALA, which typically comprises 55% of all FAs present in green vegetables. However such plant tissues are not rich in fat, and therefore, for most human diets, this source does not make a sufficient contribution to the minimum intake of ALA. In contrast, some plant oils, such as soybean, flaxseed, and rapeseed oil, as well as some kinds of dry fruits as nuts, contribute to a higher degree to ALA dietary intake, although, the absolute amount of LA is almost always superior to that of ALA [8]. Intervention studies with ALA in ω-3 PUFA-deficient patients have demonstrated marked increases in plasma concentrations of EPA [9–11]. In addition, vegans on a plant-based diet with no source of EPA and DHA in their diets have shown low but stable DHA levels in their blood [12,13]. Together, these findings preliminarily demonstrated the convertibility of ALA into EPA and DHA in humans. Such seminal evidence have been expanded using stable isotope-labeled ALA as tracer of ALA metabolism in humans [14]. These studies have shown that dietary ALA is mostly catabolized to carbon dioxide and ATP for energy production [15]. Only a small proportion of the administered ALA, estimated to be less than 5%, was metabolized to EPA and DHA [16], with greater capacity for ALA conversion in women than men [17]. ALA appears therefore to be a modest source of longer-chain ω-3 PUFAs in humans. In Europe, during the last two decades, the consumption of LA is increased by about 50%, passing from 10 to 15 g/day, while the consumption of ALA moved from 1 to 1.9 g/day [18]. Since dietary LA competes with ALA for Δ6-desaturase activity [19], the modest intakes of ALA and the high amounts of LA featuring in most western diets suggest that ALA cannot reliably replace EPA and DHA in many current diets, and therefore the direct dietary intake of EPA and DHA is by far the easiest way to increase the concentration of such FAs in human tissues [7]. Algae are the primary producers of DHA and EPA in the ecosystem, so that DHA and EPA enter the food chain through marine phytoplankton and accumulate in fish, especially oily fish (mackerel, trout, salmon, herring and sardines) [20].

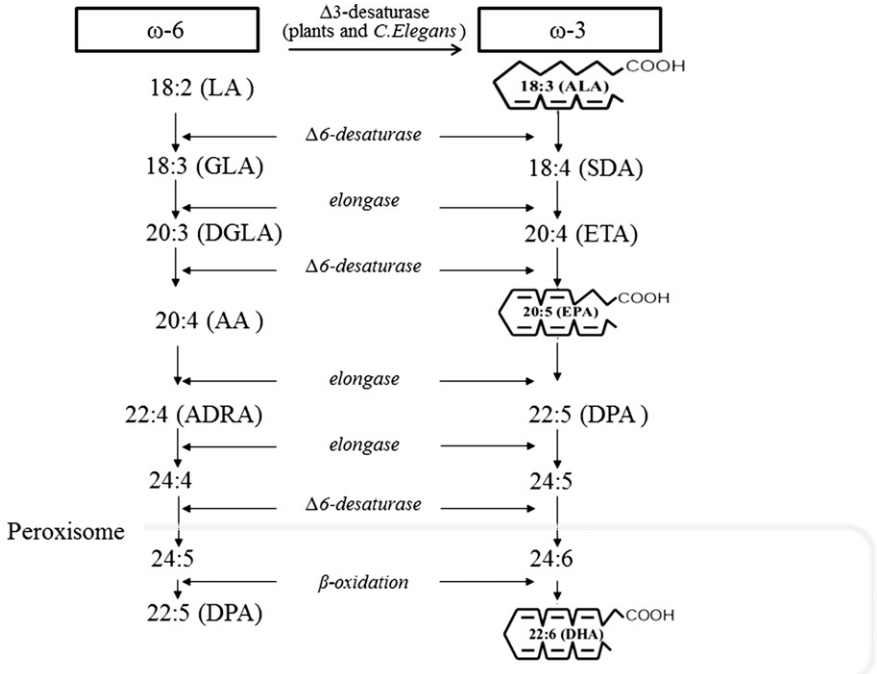

Fig. 1. Chemical structure and conversion pathway of linoleic (LA) and α-linolenic (ALA) acid into longer derivatives. In the omega-6 family, LA can be converted into γ-linolenic acid (GLA) (18: 3n-6) by Δ6-desaturase and then GLA can be elongated (by elongase) to dihomo-γ-linolenic acid (DGLA) (20: 3n-6). (DGLA) can be desaturated further by Δ5-desaturase to finally yield arachidonic acid (AA) (20: 4n-6). Using the same series of enzymes, ALA is converted into eicosapentaenoic acid (EPA) (20: 5n-3). The further conversion of EPA into docosahexaenoic acid (DHA) (22: 6n-3) involves a first addition of two carbon atoms to form docosapentaenoic acid (DPA) (22: 5n-3), of two further carbons to produce 24: 5, and a desaturation to form 24:6. The removal of two carbons from 24:6, by β-oxidation yields DHA (Sprecher's shunt). ETA, eicosatetraenoic acid; ADRA, adrenic acid.

2.2. ω-3 PUFAs in Mediterranean diets

Although high olive oil intake continues to be regarded as the main distinctive feature of Mediterranean dietary patterns, a careful itemization of all Mediterranean diet lipid components identifies at least five other "healthy lipid factors" (Table 1), among which a variable, but non-negligible, intake of ω-3 PUFAs is also a feature. Such evidence, however, raises an intriguing question: if spontaneous adherence to a typical Mediterranean diet may provide appreciable increase in plasma and tissue levels for both plant- and marine-derived ω-3 PUFAs.

In recent years, the introduction of new computational scores estimating the adherence of individuals to Mediterranean diets has substantiated the protective role of Mediterranean dietary habits in terms of reduced risk of mortality and incidence of major chronic diseases including cardiovascular disease and cancer [21]. The evaluation of adherence score, as based only on dietary data, may not accurately reflect the bioavailability of computed nutrients. Rather, the evaluation of corresponding nutritional biomarkers (as the specific nutrient itself or one or more of its direct end-products in body fluids, e.g., plasma, urine, milk or saliva) may provide more direct and integrated assessment of the nutritional status, incorporating the metabolic details of nutrient investigated. However, data on the association between patterns of adherence to Mediterranean diets and plasma ω-3 PUFA status are scarce. The first data addressing this issue date back to 1993, when Sandker et al. compared blood FA profile of participants of the Greek and Dutch cohorts of the Seven Countries Study, showing that the Greeks had 33% more ALA ($P < 0.001$) and 20% lower LA ($P < 0.001$) in their plasma [22]. Curiously, in the report by Sandker et al. there were no data regarding plasma EPA and DHA levels. Because ALA and LA are EFAs, this indicates that the traditional Greek Mediterranean diet was rich in ALA, but poor in LA and ω-6 PUFAs in general. This was not surprising, since ALA is present in a large number of plant-derived foods (including walnuts, purslane and some other green leafy vegetables) typically consumed by Mediterranean dwellers [23]. Although the total fat content of vegetables and legumes is rather low, their relative abundance in ALA, associated with the large size of the average portions and the high frequency of consumption, may result in appreciable levels of ALA intakes [24]. Thus, Mediterranean-type diets are rich in plant-derived ω-3 PUFAs, and adherence to such dietary models should guarantee adequate ALA intakes [24]. The consumption of fish and fish-derivatives in the Mediterranean countries is not geographically uniform. In certain countries, such as coastal Spain and Portugal, the intake of fish is very high, whereas it is low in other countries (e.g., Italy and Greece) [24,25]. Since the concentrations of EPA and DHA vary a lot from one fish species to another [26], fish consumption data represent only a crude estimate of the actual intake of marine ω-3 PUFAs. However, evaluation of EPA and DHA plasma concentrations in the same populations was in line with the intakes of fish, since both EPA and DHA plasma levels have resulted to be much lower among Italians living in Southern Italy than among British, Belgian or Danes [27,28]. This suggests that, at the present times, the consumption of marine ω-3 PUFAs by certain Mediterranean populations may be rather low. In spite of this, a positive association between spontaneous adherence to Mediterranean diet, as assessed by a dietary score, and plasma DHA and EPA have been recently observed in both the ATTICA [29] and the Three-City [30] studies, which independently evaluated the dietary habits of a Greek and French cohort in relation to the plasma FA contents. In particular, in the French cohort, individuals with higher Mediterranean diet adherence score, showed DHA and total ω-3 PUFAs levels 10% ($P < 0.004$) higher than individuals in the lower-score category [30]. Correspondingly, in the small intervention study by Ambring et al. [31] the administration of a Mediterranean-type diet to healthy subjects for 4 weeks resulted in a 47% increase in DHA plasma content ($P < 0.0001$) compared with an ordinary Swedish diet [31]. Although all these studies lack of the absolute quantification of plasma ω-3 PUFA content, they demonstrate that a Mediterranean-type diet ensures higher ω-3 PUFAs bioavailability than the common Western diets, and hence at least part of the protective effects exerted by Mediterranean diets may be attributed to its vegetable- and animal content of ω-3 PUFAs. Thus, both plant- and marine-derived ω-3 PUFAs may be at the moment considered as valuable mediators of protection provided by traditional Mediterranean diets.

Table 1
Traditional Mediterranean diet in terms of lipid factors.

1	Saturated fat intake: low but not very low
2	Monounsaturated fat intake: very high
3	ω-6 polyunsaturated fatty acid intake: very low
4	Plant ω-3 polyunsaturated fat intake: high but not very high
5	Marine ω-3 polyunsaturated fat intake: variable
6	Trans fatty acid intake: very low (possibly none at all)

2.3. Plant- and marine-derived ω-3 PUFAs: mechanisms of action

FAs primarily enter cell via FA protein transporters on the cell surface. Once inside the cell, they may undergo two metabolic fates: they contribute to the ATP production [32], or become substrate for the synthesis of neutral and polar lipids, thus exerting structural and signaling activities [33]. Both ω-6 and ω-3 PUFAs may be released from phospholipids through the action of cellular phospholipase-A_2 ($cPLA_2$). The released FAs may be converted through both orthodox and novel pathways to a wide array of signaling molecules. The orthodox pathways involve reactions catalyzed by cyclooxygenases (COXs) and lipoxygenases (LOXs) to biologically active eicosanoids, including prostaglandins, thromboxanes, and leukotrienes. EPA compete with arachidonic acid (AA) for COX and LOX activities, thus favoring the synthesis of 3-series prostanoids (PGE_3, PGI_3 and TXA_3), and of the 5-series LTs, comprehensively endowed with weaker pro-inflammatory and vasoconstrictive activities than those obtained from AA [34]. In addition to these classical metabolic pathways, the existence and the beneficial contribution of an "unorthodox" generation of alternative eicosanoid derivatives have been more recently appreciated. Discovered as specifically produced in the resolving exudate of a mouse dorsal air pouch using lipidomic and bioinformatic analyses [35], such alternative eicosanoids are now regarded as promising therapeutic tools for the treatment of many inflammatory-mediated disorders. From a biosynthetic point of view, such novel molecules are oxygenated metabolites derived from EPA and DHA through transcellular biosynthesis pathways that counteract excessive inflammatory responses stimulating pro-resolving mechanisms of leukocyte trafficking and non-inflammatory phagocytosis of apoptotic neutrophils by macrophages [36].

In addition, the formation of another set of compounds formed by the nitration of unsaturated FAs, called nitrolipids, has been shown to occur *in vivo* and to have potent biological actions. Such derivatives are known as nitro-fatty acids (NO_2-FA), and recent data indicate that they may act via electrophilic and receptor-mediated reactions to stimulate smooth muscle relaxation, block platelet activation, and inhibit human neutrophil function, superoxide generation, integrin and immunoglobulin expression, thus globally suppressing inflammation [37]. As evidenced in Part I of this review, Mediterranean diets are rich in nitrates, which, upon reduction to nitrites, appear to support the NO_2-FA formation under acidic gastric digestive conditions [38]. Such biochemical interaction may contribute to the healthful properties of both these components in a typical Mediterranean context.

But long-chain ω-3 PUFAs EPA and DHA may exert their effects also without undergoing metabolism [39]. Incorporation of DHA, EPA and to a lesser extend ALA into cell membranes has been shown to decrease the generation of intracellular reactive oxygen species and the following activation of redox-sensitive transcription factors, such as nuclear factor(NF)-κB, involved in the expression of a series of pro-inflammatory genes in vascular endothelial cells [39,40] and monocytes [41]. Other known signaling systems affected by ω-3 PUFAs include the toll-like receptors [42,43] and the sarcoma(Src)-family kinases [44]

overall involved in the regulation of cell growth, differentiation, cell shape, migration and survival, and specialized cell signals [45].

A recently discovered attractive ω-3 PUFA receptor is the G-protein coupled receptor 120 (GRP120) [46]. Its direct activation by EPA, DHA and ALA binding inhibits inflammatory cascades in macrophages and reverses insulin resistance in obese mice [47].

Finally, ω-3 PUFAs are also known as direct regulators of transcriptional activities that control the expression of proteins (some of which enzymes) responsible for both triglyceride assembly and FA oxidation, including sterol regulatory element-binding proteins (SREBP) and peroxisome proliferator-activated receptor (PPAR)α [20], as well as of ion channel activities that regulate cardiac rhythm [48]. Of note, the list of the mechanisms of action classically ascribed to ω-3 PUFAs has further grown in recent years by the applications of high-throughput genomic tools that allow the simultaneous comparison of thousands of genes and gene-products in a fashion not biased or restricted by *a priori* hypotheses [49]. Besides confirming the down-regulation of pro-inflammatory genes by ω-3 PUFAs [50], this approach has disclosed positive modulatory activities for previously unsuspected genes and gene products involved in the regulation of lipid metabolism and anti-oxidant enzyme systems [51–53].

Through one or more of the above mechanisms, also modest consumptions of fish or fish oils are recognized to positively affect several intermediate determinants of cardiovascular risk [4,54], as well the development of atheromatous lesions in humans [55,56] and animal models of atherosclerosis [57–61]. Less evidence however exists for a protective effect of ALA against cardiovascular disease [62]. In animal models of atherosclerosis, ALA-rich oils have been shown to confer atheroprotection, improving endothelial function and reducing plaque inflammation [63,64], although this anti-atherosclerotic effect was modest compared to DHA [65]. A recent meta-analysis of all observational studies evaluating the association between ALA intake and cardiovascular events has highlighted only a modestly lower risk of cardiovascular disease [66]. Whether ALA has beneficial effects beyond its conversion to EPA and DHA remains a matter of debate [67], given also that only limited evidence suggests that ALA may have *per se* an independent role in cardiovascular protection [68]. Further studies are therefore warranted to investigate whether ALA has independent effects on cardiovascular health and whether such effects may be modified or overcome by the intake of DHA and EPA, as suggested by recent findings [69].

3. Mediterranean diets polyphenols and vascular health

The impressive protective effects of the Mediterranean diets against cardiovascular morbidity and mortality [70,71] have long been attributed to their peculiar plant-derived foods and beverages. In particular, during the last 10 years special attention has been paid to polyphenols, a large and heterogeneous family of naturally occurring phytochemicals that are the most abundant antioxidants in our diet. Human, animal and *in vitro* studies have demonstrated that, in addition to their antioxidant properties, Mediterranean diet polyphenols possess anti-microbial, anti-inflammatory, anti-angiogenic and anti-proliferative activity, improve vascular function, and reduce intermediate clinical markers of cardiovascular diseases [72]. Special research efforts have focused on the bioactivity of polyphenols peculiar to extra-virgin olive oil. Olive oil is indeed the hallmark of the Mediterranean dietary pattern, with daily consumption between 25 and 50 mL and its main source of culinary (especially dressing) fat [72]. Nowadays, olive oil polyphenols have emerged as substantially contributing to the complex metabolic and vascular protection afforded by Mediterranean diets [72], thus becoming an area of intense current research at the crossroad of preclinical and clinical studies, with potential exploitation as functional foods, supplements or preservatives in the nutraceutical, cosmetic and food industries.

3.1. Polyphenols from olives and olive oils

Olives and olive oils, in particular virgin and extra-virgin olive oils obtained from the fruit of the olive tree (*Olea europaea*) solely by mechanical or other physical means, with a free acidity expressed as oleic acid, of not more than 0.8 and 2 g per 100 g respectively, are particularly rich sources of phenolic compounds with antioxidant and biological properties in plants as well as in animals and humans [73]. The non-glycerol or unsaponifiable fraction of olive oil (around 2% of the total weight of the oil) contains more than 230 chemical compounds, including hydrocarbons, tocopherols, fatty alcohols, 4-methylesterols, sterols, triterpenic dialcohols, polar-colored pigments and the polar phenolic compounds. The phenolic composition of olive oils varies in quantity (40–1000 mg/kg) and quality, depending on the cultivar, climate, soil composition, degree of ripeness of the fruit at harvest, agricultural and processing techniques, storage and also cooking methods [73].

Olive oil polyphenols in turn constitute a complex mixture of compounds that include phenolic acids of the benzoic (gallic, vanillic, benzoic, syringic acids) and cinnamic (cinnamic, cumaric, caffeic acids) series; phenolic alcohols, including hydroxytyrosol (HT, 3,4-dihydroxyphenylethanol), tyrosol (4-hydroxyphenylethanol), and HT glucoside; secoiridoids, which contain elenolic acid in their structure and include oleuropein and ligstroside (the ester of elenolic acid with HT or with tyrosol, respectively); lignans, such as (+)-pinoresinol and (+)-1-acetoxypinoresinol; and flavonoids (apigenin and luteolin). Notably, the olive fruit contains mainly the polar glycosides oleuropein and ligstroside. The less polar oleuropein- and ligstroside-aglycones are formed by removal of the glucose moiety from the oleuropein and ligstroside glycosides by β-glucosidase during ripening. Those aglycones and their derivatives are the most abundant phenols in olive oil. The polar compounds HT and tyrosol are the end products of hydrolysis of oleuropein- and ligstroside-aglycones or their derivatives in olives and olive oil. HT concentrations in olive oil have been reported to greatly vary from 1.55–14.42 mg/kg to 113.7–381.2 mg/kg [74].

Of the various phenolic constituents of olive oil, HT and its parent compound oleuropein seem to be the most important, and feature powerful antioxidant properties *in vitro* and *in vivo* due to their *ortho*-diphenolic structure (Fig. 2) [75,76].

3.2. Dietary intake and bioavailability of olive oil polyphenols

Using the recent development of a new database on the content of polyphenols in foods (fruits, vegetables, beverages, cereals, oils, nuts and seeds, cocoa, and legumes), the dietary intake and the major Mediterranean food sources of polyphenols have been determined in a Spanish population at high cardiovascular risk, in the PREDIMED cohort [77]. The PREDIMED study is an ongoing cardiovascular primary prevention trial conducted in Spain in a high-risk population, comparing two energy-unrestricted Mediterranean diets enriched with extra-virgin olive oil (1 L/week) or mixed nuts (30 g/die) with a low-fat control diet on a composite endpoint of cardiovascular death, myocardial

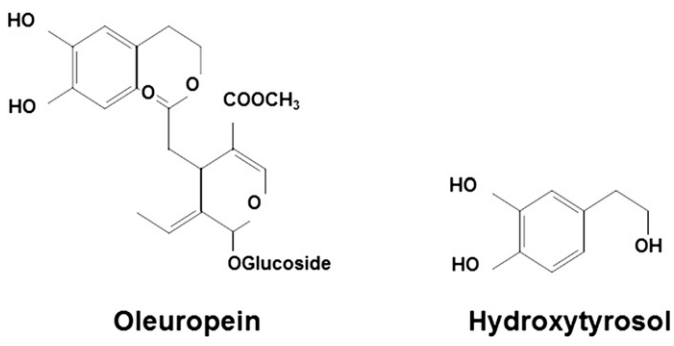

Fig. 2. Chemical structures of oleuropein glucoside and its derivative hydroxytyrosol.

infarction and stroke ('primary endpoint') [78]. The estimated mean total polyphenol intake in the PREDIMED Mediterranean diets was 820 ± 323 mg/day, with olives and olive oils providing 11% (90.4 mg/day) of total polyphenol intake. The specific contribution of olives and olive oil to the total polyphenol intake gives the Spanish population a particular phenolic profile that is also likely to be characteristic in other Mediterranean countries.

Regarding bioavailability, previous human studies have provided evidence that olive oil polyphenols are readily and dose-dependently absorbed (55–66% of the total ingested amount) and excreted in urine after consumption of real-life doses of virgin olive oils, with phenol compounds reaching the maximal concentration in plasma and urine (0.1–1 μmol/L) between 1 and 2 h after olive oil ingestion [79]. Oleuropein glycoside and oleuropein- and ligstroside-aglicones are converted to HT and tyrosol, so that substantially more free HT and tyrosol enter the small intestine. The ingested olive oil phenols are present in plasma and urine in the free form and predominantly in conjugated forms (glucuronide and sulfate conjugates) and in the O-methylated form, suggesting an extensive first pass intestinal/hepatic metabolism [80]. As a consequence, olive oil polyphenol bioactivity is likely to be derived mainly from its biological metabolites, but studies in this field are still scarce and have so far shown contrasting results [81]. Importantly, urinary or plasma tyrosol, HT and their metabolites have been used as reliable biomarkers of olive oil intake in human intervention studies [82,3].

3.3. Olive oil polyphenols and cardiovascular protection: effects on atherothrombotic risk factors

A great number of studies has been devoted to characterizing the effects of olive oil polyphenols on atherothrombotic risk factors, such as plasma lipid profile, oxidative stress, endothelial dysfunction, hypertension, platelet aggregation, diabetes, and inflammation [83]. In November 2004, the US Food and Drug Administration approved a health claim for olive oil consumption (23 g/day) on the basis of its MUFA content [84], that had consistently demonstrated to benefit plasma lipid profile in clinical trials. More recently, the European Food Safety Authority (EFSA) has released a health claim about the role of olive oil polyphenols (5 mg/day), i.e. HT, tyrosol, and their conjugated forms, in protecting low-density lipoprotein (LDL) from oxidation *in vivo* [85]. LDL oxidation is considered a key pathogenetic event in atherosclerosis and subsequent coronary heart disease development. A key supportive study was the Effect of Olive Oil on Oxidative Damage in European Populations (EUROLIVE) study, a randomized clinical intervention trial assessing the effect of sustained daily doses of olive oil, evaluated as a function of its phenolic content, on the lipid profile and markers of oxidative damage, as cardiovascular risk factors [76]. In this trial, 200 healthy volunteers were randomized to three-week consecutive intervention periods of olive oil administration (25 mL/day) with high (366 mg/kg of olive oil), medium (164 mg/kg), and low (2.7 mg/kg) polyphenol content. The results have shown that olive oil phenols, which are able to bind human LDL, are significantly associated with lower circulating concentrations of oxidative stress markers, including cholesterol-conjugated dienes, hydroxy-fatty acids and products of DNA oxidative damage, and with an improvement of the lipid profile, including an increase of high-density lipoprotein (HDL) levels, a decrease of total cholesterol/HDL ratio, and a decrease of triglyceride levels [76], providing a convincing evidence for greater benefits of olive oil polyphenols on these cardiovascular risk factors than that provided by MUFA and other minor components of olive oil. Decreased levels of oxidized LDL were also shown after three-week supplementation of healthy subjects with HT-enriched sunflower oil (45–50 mg/day of HT), pointing to a significant contribution by HT to the observed cardiovascular benefits of olive oil polyphenols [86]. Similar results were obtained in patients with stable coronary heart disease [87].

Other recent lines of evidence in humans indicate that olive oil with a high polyphenol content improves endothelial dysfunction in different clinical settings [88], with consequent improvement in endothelial-dependent vasodilatation and blood pressure [87,89]. Oxidative stress, through superoxide anion generation, decreases NO availability causing endothelial dysfunction. The observed benefit of olive oil polyphenols on ischemic reactive hyperemia was accompanied by a decrease in oxidative stress markers and an increase in NO metabolites, indicating that the antioxidant effect could account for the protective effect of olive oil polyphenols on vascular endothelial function [88,89].

One of the main properties of olive oil polyphenols contributing to their beneficial vascular effects is their anti-inflammatory activities. Early reports showed that oleocanthal, a virgin olive oil phenol, possesses a strong anti-inflammatory effect, comparable to that of ibuprofen and mediated by inhibition of cycooxygenase (COX)-1 and -2 activities [90]. Other *in vitro* studies demonstrated that HT and oleuropein, at nutritionally relevant concentrations, inhibited endothelial activation and endothelium–monocyte adhesion as well as monocyte/macrophage function [91–93]. The anti-inflammatory action has been confirmed in humans, in whom a decrease in inflammatory markers such as thromboxanes, leukotrienes, cytokines, C-reactive protein, and soluble adhesion molecules has been observed after the intake of phenol-rich olive oil [94–96].

Olive oil polyphenols, in particular HT, have also been shown to beneficially impact the hemostatic profile, showing anti-platelet and anti-thrombotic properties both *in vitro* [97] and *in vivo* [82], and to possess some anti-diabetic and anti-obesity effects [98].

Overall, these protective properties by olive oil polyphenols, through favorable modifications of atherothrombotic risk factors and direct effects on inflammatory and vascular cells, support the clinically proven cardioprotection provided by Mediterranean diets and virgin olive oil.

3.4. Nutrigenomic effects of olive oil polyphenols

Up to now, the evidence for the contribution of olive oil polyphenols to the nutrigenomic effects of Mediterranean diet or olive oil is provided by human nutritional intervention studies comparing similar olive oils with different polyphenol contents in real-life conditions. Current evidence indicates that, besides reducing oxidative stress markers, inflammation and atherosclerotic risk factors (ox-LDL, diabetes, hypertension), olive oil polyphenols induce specific favorable changes in the expression profile of genes involved in atherosclerosis, inflammation, and oxidative stress [99–101]. For example, recent human nutrigenomic studies have shown that a sustained consumption of a Mediterranean diet enriched with virgin olive oil reduces the pro-inflammatory and pro-atherogenic responses of peripheral blood mononuclear cells in comparison with a Western diet rich in saturated fat [93,94], or with a traditional Mediterranean diet supplemented with a low-fat diet [95]. In a transcriptomic study, 90 healthy volunteers were randomized to a three-month intervention consisting of either a traditional Mediterranean diet with virgin olive oil (1 L/week), a traditional Mediterranean diet with washed olive oil containing a lower polyphenol content (55 and 328 mg/kg), or a habitual diet [99]. The traditional Mediterranean diets significantly downregulated several genes related to inflammation and oxidative stress in peripheral blood mononuclear cells, with concomitant decreases in markers of lipid oxidative damage and systemic inflammation. Interestingly, these effects were particularly observed when a traditional Mediterranean diet was supplemented with virgin olive oil, thus pointing out to the role of olive oil polyphenols in decreasing the expression of atherosclerosis-related genes in the context of a Mediterranean diet [99]. With a less-restricted, non-hypothesis driven scope, Camargo et al. [100] performed a post-prandial gene expression microarray analysis on peripheral blood mononuclear cells from 20 patients with the metabolic syndrome after the intake of virgin olive oil-based breakfasts with a high (398 mg/kg of olive oil) and low (70 mg/kg of olive oil) phenolic content. The intake of a high polyphenol content olive oil (40 mL) modified the expression of pro-inflammatory

genes linked to atherosclerosis, obesity, dyslipidemia and type 2 diabetes mellitus, thereby promoting a lesser inflammatory profile in peripheral blood mononuclear cells [100].

In accordance with results from human intervention trials, studies in cell model systems relevant to cardiovascular disease, such as vascular endothelial cells and monocytes/macrophages, reinforce the nutrigenomic effects of olive oil polyphenols as a mechanism for their vasculoprotective role. Olive oil polyphenols, including HT and oleuropein at nutritionally relevant concentrations, downregulate the gene expression of adhesion molecules, chemoattractants, matrix metalloproteinase, and pro-inflammatory enzymes [92,93,101]. Other studies have shown that HT upregulates the gene expression of antioxidant/detoxifying enzymes in endothelial cells [102], as well as of the histone deacetylase sirtuin (SIRT) 1, an epigenetic modifier, in the heart tissue of a mouse model of accelerated senescence [103].

4. Conclusions

The high dietary intake of inorganic nitrates and MUFA (as described in the first part of this review), as well as ω-3PUFAs and olive oil polyphenols (as described here above) substantially contributes to the protection provided by the traditional Mediterranean diets against chronic degenerative diseases, including cancer and cardiovascular diseases. Omega-3PUFAs and olive oil polyphenols can exert protective effects not only through the scavenging of reactive oxygen species, but also by modulating signal transduction pathways and gene expression. However, foods or nutrients are not eaten in isolation but consumed in combination, in the form of a global dietary approach, where additive/synergistic interactions among nutrients and foods occur. As an example, mutual interactions between polyphenols and ω-3 PUFAs have recently been observed. The addition of polyphenols during the active digestion of fatty fish may limit the formation of ω-3 PUFAs oxidation products in the small intestine and therefore promote the intestinal uptake of beneficial unoxidized ω-3 PUFAs [104]. Mediterranean diets should be regarded as a combination of healthful individual components promoting overall health.

Therefore, nutritional research and health policy should focus their attention to the preservation and promotion of traditional Mediterranean-style diets, also in light of their recent progressive deviation from the initially identified compositions in the 1960s, deviations that may in turn negatively influence the health status of Mediterranean populations [105].

References

[1] de Lorgeril M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, et al. Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. Lancet 1994;343:1454–9.
[2] Tulipani S, Urpi-Sarda M, Garcia-Villalba R, Rabassa M, Lopez-Uriarte P, Bullo M, et al. Urolithins are the main urinary microbial-derived phenolic metabolites discriminating a moderate consumption of nuts in free-living subjects with diagnosed metabolic syndrome. J Agric Food Chem 2012;60:8930–40.
[3] Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, et al. Primary prevention of cardiovascular disease with a Mediterranean diet. N Engl J Med 2013;368:1279–90.
[4] De Caterina R. n-3 fatty acids in cardiovascular disease. N Engl J Med 2011;364:2439–50.
[5] Burr GO, Burr MM. Nutrition classics from The Journal of Biological Chemistry 82:345-67, 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. Nutr Rev 1973;31:248–9.
[6] Yamazaki K, Fujikawa M, Hamazaki T, Yano S, Shono T. Comparison of the conversion rates of alpha-linolenic acid (18:3(n - 3)) and stearidonic acid (18:4(n-3)) to longer polyunsaturated fatty acids in rats. Biochim Biophys Acta 1992;1123:18–26.
[7] Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food Nutr Pap 2010;91:1–166.
[8] Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol 2009;77:937–46.
[9] Holman RT, Johnson SB, Hatch TF. A case of human linolenic acid deficiency involving neurological abnormalities. Am J Clin Nutr 1982;35:617–23.
[10] Bjerve KS, Fischer S, Wammer F, Egeland T. alpha-Linolenic acid and long-chain omega-3 fatty acid supplementation in three patients with omega-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. Am J Clin Nutr 1989;49:290–300.
[11] Bjerve KS, Mostad IL, Thoresen L. Alpha-linolenic acid deficiency in patients on long-term gastric-tube feeding: estimation of linolenic acid and long-chain unsaturated n-3 fatty acid requirement in man. Am J Clin Nutr 1987;45:66–77.
[12] Phinney SD, Odin RS, Johnson SB, Holman RT. Reduced arachidonate in serum phospholipids and cholesteryl esters associated with vegetarian diets in humans. Am J Clin Nutr 1990;51:385–92.
[13] Rosell MS, Lloyd-Wright Z, Appleby PN, Sanders TA, Allen NE, Key TJ. Long-chain n-3 polyunsaturated fatty acids in plasma in British meat-eating, vegetarian, and vegan men. Am J Clin Nutr 2005;82:327–34.
[14] Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. Curr Opin Clin Nutr Metab Care 2004;7:137–44.
[15] Vermunt SH, Mensink RP, Simonis MM, Hornstra G. Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha-linolenic acid. Lipids 2000;35:137–42.
[16] Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. Curr Opin Clin Nutr Metab Care 2002;5:127–32.
[17] Burdge GC, Wootton SA. Conversion of alpha-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. Br J Nutr 2002;88:411–20.
[18] Sanders TA. Polyunsaturated fatty acids in the food chain in Europe. Am J Clin Nutr 2000;71:176S–8S.
[19] Emken EA, Adlof RO, Gulley RM. Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochim Biophys Acta 1994;1213:277–88.
[20] Massaro M, Scoditti E, Carluccio MA, De Caterina R. Basic mechanisms behind the effects of n-3 fatty acids on cardiovascular disease. Prostaglandins Leukot Essent Fat Acids 2008;79:109–15.
[21] Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;348:2599–608.
[22] Sandker GW, Kromhout D, Aravanis C, Bloemberg BP, Mensink RP, Karalias N, et al. Serum cholesteryl ester fatty acids and their relation with serum lipids in elderly men in Crete and The Netherlands. Eur J Clin Nutr 1993;47:201–8.
[23] Simopoulos AP. Omega-3 fatty acids and antioxidants in edible wild plants. Biol Res 2004;37:263–77.
[24] Galli C, Marangoni F. N-3 fatty acids in the Mediterranean diet. Prostaglandins Leukot Essent Fatty Acids 2006;75:129–33.
[25] Food and Agriculture Organization of United Nations (FAO). Fish consumption and production in Mediterranean countries. In: FAO, corporate document repository 1987.
[26] Lloret J. Human health benefits supplied by Mediterranean marine biodiversity. Mar Pollut Bull 2010;60:1640–6.
[27] de Lorgeril M, Salen P. Mediterranean diet and n-3 fatty acids in the prevention and treatment of cardiovascular disease. J Cardiovasc Med (Hagerstown) 2007;8(Suppl. 1):S38–41.
[28] Saadatian-Elahi M, Slimani N, Chajes V, Jenab M, Goudable J, Biessy C, et al. Plasma phospholipid fatty acid profiles and their association with food intakes: results from a cross-sectional study within the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr 2009;89:331–46.
[29] Panagiotakos D, Kalogeropoulos N, Pitsavos C, Roussinou G, Palliou K, Chrysohoou C, et al. Validation of the MedDietScore via the determination of plasma fatty acids. Int J Food Sci Nutr 2009;60(Suppl. 5):168–80.
[30] Feart C, Torres MJ, Samieri C, Jutand MA, Peuchant E, Simopoulos AP, et al. Adherence to a Mediterranean diet and plasma fatty acids: data from the Bordeaux sample of the Three-City study. Br J Nutr 2011;106:149–58.
[31] Ambring A, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. Am J Clin Nutr 2006;83:575–81.
[32] Burdge GC. Metabolism of alpha-linolenic acid in humans. Prostaglandins Leukot Essent Fatty Acids 2006;75:161–8.
[33] Das UN. Essential fatty acids—a review. Curr Pharm Biotechnol 2006;7:467–82.
[34] Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie 2009;91:791–5.
[35] Serhan CN, Clish CB, Brannon J, Colgan SP, Chiang N, Gronert K. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J Exp Med 2000;192:1197–204.
[36] Lee HN, Surh YJ. Therapeutic potential of resolvins in the prevention and treatment of inflammatory disorders. Biochem Pharmacol 2012;84:1340–50.
[37] Freeman BA, Baker PR, Schopfer FJ, Woodcock SR, Napolitano A, d'Ischia M. Nitro-fatty acid formation and signaling. J Biol Chem 2008:1–16.
[38] Fazzari M, Trostchansky A, Schopfer FJ, Salvatore SR, Sanchez-Calvo B, Vitturi D, et al. Olives and olive oil are sources of electrophilic fatty acid nitroalkenes. PLoS One 2014;9:e84884.
[39] Massaro M, Habib A, Lubrano L, Del Turco S, Lazzerini G, Bourcier T, et al. The omega-3 fatty acid docosahexaenoate attenuates endothelial cyclooxygenase-2 induction through both NADP(H) oxidase and PKC epsilon inhibition. Proc Natl Acad Sci U S A 2006;103:15184–9.
[40] De Caterina R, Cybulsky MI, Clinton SK, Gimbrone Jr MA, Libby P. The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. Arterioscler Thromb 1994;14:1829–36.

[41] Zhao G, Etherton TD, Martin KR, Vanden Heuvel JP, Gillies PJ, West SG, et al. Anti-inflammatory effects of polyunsaturated fatty acids in THP-1 cells. Biochem Biophys Res Commun 2005;336:909–17.
[42] Hsueh HW, Zhou Z, Whelan J, Allen KG, Moustaid-Moussa N, Kim H, et al. Stearidonic and eicosapentaenoic acids inhibit interleukin-6 expression in ob/ob mouse adipose stem cells via Toll-like receptor-2-mediated pathways. J Nutr 2011;141:1260–6.
[43] Lee JY, Zhao L, Hwang DH. Modulation of pattern recognition receptor-mediated inflammation and risk of chronic diseases by dietary fatty acids. Nutr Rev 2010; 68:38–61.
[44] Chen W, Jump DB, Esselman WJ, Busik JV. Inhibition of cytokine signaling in human retinal endothelial cells through modification of caveolae/lipid rafts by docosahexaenoic acid. Invest Ophthalmol Vis Sci 2007;48:18–26.
[45] Parsons SJ, Parsons JT. Src family kinases, key regulators of signal transduction. Oncogene 2004;23:7906–9.
[46] Cornall LM, Mathai ML, Hryciw DH, McAinch AJ. GPR120 agonism as a countermeasure against metabolic diseases. Drug Discov Today 2014;19:670–9.
[47] Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell 2010;142:687–98.
[48] Billman GE. The effects of omega-3 polyunsaturated fatty acids on cardiac rhythm: a critical reassessment. Pharmacol Ther 2013;140:53–80.
[49] Trevino V, Falciani F, Barrera-Saldana HA. DNA microarrays: a powerful genomic tool for biomedical and clinical research. Mol Med 2007;13:527–41.
[50] Bouwens M, van de Rest O, Dellschaft N, Bromhaar MG, de Groot LC, Geleijnse JM, et al. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 2009;90:415–24.
[51] Schmidt S, Willers J, Stahl F, Mutz KO, Scheper T, Hahn A, et al. Regulation of lipid metabolism-related gene expression in whole blood cells of normo- and dyslipidemic men after fish oil supplementation. Lipids Health Dis 2012;11: 172.
[52] Schmidt S, Stahl F, Mutz KO, Scheper T, Hahn A, Schuchardt JP. Different gene expression profiles in normo- and dyslipidemic men after fish oil supplementation: results from a randomized controlled trial. Lipids Health Dis 2012;11:105.
[53] Schmidt S, Stahl F, Mutz KO, Scheper T, Hahn A, Schuchardt JP. Transcriptome-based identification of antioxidative gene expression after fish oil supplementation in normo- and dyslipidemic men. Nutr Metab (Lond) 2012;9:45.
[54] Mozaffarian D, Rimm EB. Fish intake, contaminants, and human health: evaluating the risks and the benefits. JAMA 2006;296:1885–99.
[55] Thies F, Garry JM, Yaqoob P, Rerkasem K, Williams J, Shearman CP, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. Lancet 2003;361:477–85.
[56] Mita T, Watada H, Ogihara T, Nomiyama T, Ogawa O, Kinoshita J, et al. Eicosapentaenoic acid reduces the progression of carotid intima-media thickness in patients with type 2 diabetes. Atherosclerosis 2007;191:162–7.
[57] Li S, Sun Y, Liang CP, Thorp EB, Han S, Jehle AW, et al. Defective phagocytosis of apoptotic cells by macrophages in atherosclerotic lesions of ob/ob mice and reversal by a fish oil diet. Circ Res 2009;105:1072–82.
[58] Matsumoto M, Sata M, Fukuda D, Tanaka K, Soma M, Hirata Y, et al. Orally administered eicosapentaenoic acid reduces and stabilizes atherosclerotic lesions in ApoE-deficient mice. Atherosclerosis 2008;197:524–33.
[59] Wan JB, Huang LL, Rong R, Tan R, Wang J, Kang JX. Endogenously decreasing tissue n-6/n-3 fatty acid ratio reduces atherosclerotic lesions in apolipoprotein E-deficient mice by inhibiting systemic and vascular inflammation. Arterioscler Thromb Vasc Biol 2010;30:2487–94.
[60] Wang S, Wu D, Matthan NR, Lamon-Fava S, Lecker JL, Lichtenstein AH. Reduction in dietary omega-6 polyunsaturated fatty acids: eicosapentaenoic acid plus docosahexaenoic acid ratio minimizes atherosclerotic lesion formation and inflammatory response in the LDL receptor null mouse. Atherosclerosis 2009;204: 147–55.
[61] Zampolli A, Bysted A, Leth T, Mortensen A, De Caterina R, Falk E. Contrasting effect of fish oil supplementation on the development of atherosclerosis in murine models. Atherosclerosis 2006;184:78–85.
[62] Wendland E, Farmer A, Glasziou P, Neil A. Effect of alpha linolenic acid on cardiovascular risk markers: a systematic review. Heart 2006;92:166–9.
[63] Dupasquier CM, Dibrov E, Kneesh AL, Cheung PK, Lee KG, Alexander HK, et al. Dietary flaxseed inhibits atherosclerosis in the LDL receptor-deficient mouse in part through antiproliferative and anti-inflammatory actions. Am J Physiol Heart Circ Physiol 2007;293:H2394–402.
[64] Winnik S, Lohmann C, Richter EK, Schafer N, Song WL, Leiber F, et al. Dietary alpha-linolenic acid diminishes experimental atherogenesis and restricts T cell-driven inflammation. Eur Heart J 2011;32:2573–84.
[65] Degirolamo C, Kelley KL, Wilson MD, Rudel LL. Dietary n-3 LCPUFA from fish oil but not alpha-linolenic acid-derived LCPUFA confers atheroprotection in mice. J Lipid Res 2010;51:1897–905.
[66] Pan A, Chen M, Chowdhury R, Wu JH, Sun Q, Campos H, et al. alpha-Linolenic acid and risk of cardiovascular disease: a systematic review and meta-analysis. Am J Clin Nutr 2012;96:1262–73.
[67] Harris WS. Cardiovascular risk and alpha-linolenic acid: can Costa Rica clarify? Circulation 2008;118:323–4.
[68] Eussen SR, Geleijnse JM, Giltay EJ, Rompelberg CJ, Klungel OH, Kromhout D. Effects of n-3 fatty acids on major cardiovascular events in statin users and non-users with a history of myocardial infarction. Eur Heart J 2012;33:1582–8.
[69] Mozaffarian D, Ascherio A, Hu FB, Stampfer MJ, Willett WC, Siscovick DS, et al. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. Circulation 2005;111:157–64.
[70] de Lorgeril M, Salen P, Martin JL, Monjaud I, Dalaye 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: 779–85.
[71] Estruch R, Ros E, Martinez-Gonzalez MA. Mediterranean diet for primary prevention of cardiovascular disease. N Engl J Med 2013;369:676–7.
[72] Martin-Pelaez S, Covas MI, Fito M, Kusar A, Pravst I. Health effects of olive oil polyphenols: recent advances and possibilities for the use of health claims. Mol Nutr Food Res 2013;57:760–71.
[73] Carluccio MA, Massaro M, Scoditti E, De Caterina R. Vasculoprotective potential of olive oil components. Mol Nutr Food Res 2007;51:1225–34.
[74] Granados-Principal S, Quiles JL, Ramirez-Tortosa CL, Sanchez-Rovira P, Ramirez-Tortosa MC. Hydroxytyrosol: from laboratory investigations to future clinical trials. Nutr Rev 2010;68:191–206.
[75] Visioli F, Bellomo G, Galli C. Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Commun 1998;247:60–4.
[76] Covas MI, Nyyssonen K, Poulsen HE, Kaikkonen J, Zunft HJ, Kiesewetter H, et al. The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. Ann Intern Med 2006;145:333–41.
[77] Tresserra-Rimbau A, Medina-Remon A, Perez-Jimenez J, Martinez-Gonzalez MA, Covas MI, Corella D, et al. Dietary intake and major food sources of polyphenols in a Spanish population at high cardiovascular risk: the PREDIMED study. Nutr Metab Cardiovasc Dis 2013;23:953–9.
[78] Estruch R, Martinez-Gonzalez MA, Corella D, Salas-Salvado J, Ruiz-Gutierrez V, Covas MI, et al. Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. Ann Intern Med 2006;145:1–11.
[79] Suarez M, Valls RM, Romero MP, Macia A, Fernandez S, Giralt M, et al. Bioavailability of phenols from a phenol-enriched olive oil. Br J Nutr 2011;106:1691–701.
[80] Visioli F, De La Lastra CA, Andres-Lacueva C, Aviram M, Calhau C, Cassano A, et al. Polyphenols and human health: a prospectus. Crit Rev Food Sci Nutr 2011;51: 524–46.
[81] Khymenets O, Fito M, Tourino S, Munoz-Aguayo D, Pujadas M, Torres JL, et al. Antioxidant activities of hydroxytyrosol main metabolites do not contribute to beneficial health effects after olive oil ingestion. Drug Metab Dispos 2010;38:1417–21.
[82] Ruano J, Lopez-Miranda J, de la Torre R, Delgado-Lista J, Fernandez J, Caballero J, et al. Intake of phenol-rich virgin olive oil improves the postprandial prothrombotic profile in hypercholesterolemic patients. Am J Clin Nutr 2007;86: 341–6.
[83] Lou-Bonafonte JM, Arnal C, Navarro MA, Osada J. Efficacy of bioactive compounds from extra virgin olive oil to modulate atherosclerosis development. Mol Nutr Food Res 2012;56:1043–57.
[84] Administration, U.F.a.D. November 1, 2004.
[85] European Food Safety Authority (EFSA), Panel on Dietetic Products, Nutrition and Allergies. Scientific Opinion on the substantiation of health claims related to polyphenols in olive. EFSA J 2011;9:2033–58.
[86] Vazquez-Velasco M, Esperanza Diaz L, Lucas R, Gomez-Martinez S, Bastida S, Marcos A, et al. Effects of hydroxytyrosol-enriched sunflower oil consumption on CVD risk factors. Br J Nutr 2011;105:1448–52.
[87] Fito M, Cladellas M, de la Torre R, Marti J, Alcantara M, Pujadas-Bastardes M, et al. Antioxidant effect of virgin olive oil in patients with stable coronary heart disease: a randomized, crossover, controlled, clinical trial. Atherosclerosis 2005;181: 149–58.
[88] Ruano J, Lopez-Miranda J, Fuentes F, Moreno JA, Bellido C, Perez-Martinez P, et al. Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. J Am Coll Cardiol 2005;46:1864–8.
[89] Moreno-Luna R, Munoz-Hernandez R, Miranda ML, Costa AF, Jimenez-Jimenez L, Vallejo-Vaz AJ, et al. Olive oil polyphenols decrease blood pressure and improve endothelial function in young women with mild hypertension. Am J Hypertens 2012;25:1299–304.
[90] Beauchamp GK, Keast RS, Morel D, Lin J, Pika J, Han Q, et al. Phytochemistry: ibuprofen-like activity in extra-virgin olive oil. Nature 2005;437:45–6.
[91] Carluccio MA, Siculella L, Ancora MA, Massaro M, Scoditti E, Storelli C, et al. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation - Antiatherogenic properties of Mediterranean diet phytochemicals. Arterioscler Thromb Vasc Biol 2003;23:622–9.
[92] Carluccio MA, Ancora MA, Massaro M, Carluccio M, Scoditti E, Distante A, et al. Homocysteine induces VCAM-1 gene expression through NF-kappa B and NAD(P)H oxidase activation: protective role of Mediterranean diet polyphenolic antioxidants. Am J Physiol Heart Circ Physiol 2007;293:H2344–54.
[93] Scoditti E, Nestola A, Massaro M, Calabriso N, Storelli C, De Caterina R, et al. Hydroxytyrosol suppresses MMP-9 and COX-2 activity and expression in activated human monocytes via PKCalpha and PKCbeta1 inhibition. Atherosclerosis 2014; 232:17–24.
[94] Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, et al. Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. Eur J Nutr 2005;44:121–7.
[95] Pacheco YM, Bemudez B, Lopez S, Abia R, Villar J, Muriana FJ. Minor compounds of olive oil have postprandial anti-inflammatory effects. Br J Nutr 2007;98:260–3.
[96] Fito M, Cladellas M, de la Torre R, Marti J, Munoz D, Schroder H, et al. Anti-inflammatory effect of virgin olive oil in stable coronary disease patients: a randomized, crossover, controlled trial. Eur J Clin Nutr 2008;62:570–4.
[97] Petroni A, Blasevich M, Salami M, Papini N, Montedoro GF, Galli C. Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. Thromb Res 1995;78:151–60.
[98] de Bock M, Derraik JG, Brennan CM, Biggs JB, Morgan PE, Hodgkinson SC, et al. Olive (Olea europaea L.) leaf polyphenols improve insulin sensitivity in middle-aged

overweight men: a randomized, placebo-controlled, crossover trial. PLoS One 2013;8:e57622.
[99] Konstantinidou V, Covas MI, Munoz-Aguayo D, Khymenets O, de la Torre R, Saez G, et al. In vivo nutrigenomic effects of virgin olive oil polyphenols within the frame of the Mediterranean diet: a randomized controlled trial. FASEB J 2010;24:2546–57.
[100] Camargo A, Ruano J, Fernandez JM, Parnell LD, Jimenez A, Santos-Gonzalez M, et al. Gene expression changes in mononuclear cells in patients with metabolic syndrome after acute intake of phenol-rich virgin olive oil. BMC Genomics 2010;11:253.
[101] Scoditti E, Calabriso N, Massaro M, Pellegrino M, Storelli C, Martines G, et al. Mediterranean diet polyphenols reduce inflammatory angiogenesis through MMP-9 and COX-2 inhibition in human vascular endothelial cells: a potentially protective mechanism in atherosclerotic vascular disease and cancer. Arch Biochem Biophys 2012;527:81–9.
[102] Zrelli H, Matsuoka M, Kitazaki S, Zarrouk M, Miyazaki H. Hydroxytyrosol reduces intracellular reactive oxygen species levels in vascular endothelial cells by upregulating catalase expression through the AMPK-FOXO3a pathway. Eur J Pharmacol 2011;660:275–82.
[103] Bayram B, Nikolai S, Huebbe P, Ozcelik B, Grimm S, Grune T, et al. Biomarkers of oxidative stress, antioxidant defence and inflammation are altered in the senescence-accelerated mouse prone 8. Age 2013;35:1205–17.
[104] Maestre R, Douglass JD, Kodukula S, Medina I, Storch J. Alterations in the intestinal assimilation of oxidized PUFAs are ameliorated by a polyphenol-rich grape seed extract in an in vitro model and Caco-2 cells. J Nutr 2013;143:295–301.
[105] Bach-Faig A, Berry EM, Lairon D, Reguant J, Trichopoulou A, Dernini S, et al. Mediterranean diet pyramid today. Science and cultural updates. Public Health Nutr 2011;14:2274–84.